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TECHNICAL REVIEW AND APPROVAL

AFAMRL-TR-84-052

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

The voluntary informed consent of the subjects used in this research was obtained as required by Air Force Regulation 169-3.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

CHARLES BATES, JR.
Director, Human Engineering Division
Air Force Aerospace Medical Research Laboratory

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

1a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution is unlimited.
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S) AFAMRL-TR-84-052
6a. NAME OF PERFORMING ORGANIZATION Midwest Research Institute 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION AFAMRL/HEG
6c. ADDRESS (City, State and ZIP Code) 425 Volker Blvd. Kansas City, Missouri 64110	7b. ADDRESS (City, State and ZIP Code) Wright-Patterson AFB, Ohio 45433-6573
8a. NAME OF FUNDING/SPONSORING ORGANIZATION 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER F33615-80-C-0606
8c. ADDRESS (City, State and ZIP Code)	10. SOURCE OF FUNDING NOS.

PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT NO.
62202F	2729	07	05

11. TITLE (Include Security Classification) EFFECTS OF PYRIDOSTIGMINE ON PSYCHOMOTOR AND VISUAL PERFORMANCE

12. PERSONAL AUTHOR(S)
Graham, Charles, Ph.D. and Cook, Mary R., Ph.D.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Yr., Mo., Day)	15. PAGE COUNT
Final	FROM Apr 83 TO Aug 84	1984 September	93

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB. GR.	
05	10		Pyridostigmine
06	15		Psychomotor Performance
			Visual Performance

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Pyridostigmine is a reversible anticholinesterase inhibitor long used in the medical treatment of the neuromuscular disorder myasthenia gravis. Due to the site and the reversible nature of its action, this drug is currently being considered by the USAF for field use as a pretreatment medication to aid pilot survival in the event of a chemical attack. Medical reports indicate that high daily oral doses (600 mg/day) are well tolerated in patient populations. Recent NATO studies also suggest that lower dose regimens (30 mg, 3 x day, 30 days) can provide enhanced survival protection in nonclinical populations, with only minor gastric upset reported in a few individuals.

Although health risks appear minimal, there exists a significant need to evaluate the impact of the drug on human functions important in pilot operations. The present study addressed this need. A double-blind, cross-over experimental design was used to evaluate the effects of an oral regimen of pyridostigmine (30 mg, 3 x day, 5 days) on the performance, physiology, and subjective state of 24 paid, male volunteers.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT. ☐ DTIC USERS ☐	UNCLASSIFIED

22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE NUMBER (Include Area Code)	22c. OFFICE SYMBOL
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DD FORM 1473, 83 APR EDITION OF 1 JAN 73 IS OBSOLETE.

PREFACE

This final report describes the methods employed and the results obtained in a research project conducted by Midwest Research Institute under Air Force Contract No. F33615-80-C-0606, "Effects of Pyridostigmine on Psychomotor and Visual Performance." during the perod April 1, 1983, to August 31, 1984. The COTR was Mr. Ronald E. Yates (AFAMRL/HET). The study was performed in the Life Sciences Department, Dr. Sophia Fotopoulos, Director. Drs. Charles Craham and Mary R. Cook were co-principal investigators. Ms. Mary Gerkovich served as project leader. The authors wish to thank: Mr. Harvey D. Cohen, Mr. James Phelps, Ms. Eva Koontz, Ms. Catherine Martin, Ms. Kathleen Coggins, Ms. Barbara Fears, Mr. Howard Lang, and Mr. Ralph Miller for their help in the performance of the study; Drs. Paul Diederich and Bruce H. Salvaggio for conducting the medical examinations; and Dr. Sophia Fotopoulos for her constructive review of this report. For the protection of human subjects, the investigators adhered to policies of applicable Federal Law 45CFR46.

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SUMMARY

Development of pretreatment and prophylactic drugs to aid survival in a chemical attack must include evaluation of the impact of such drugs on human functions important in military operations. This study evaluated the effects of pyridostigmine on human performance, physiology, and subjective state. An oral regimen (30 mg, 3 times per day, 5 days) was administered to 24 paid, male volunteers using a double-blind, cross-over design. Prior to drug testing, a medical examination and baseline measures were obtained, and subjects were trained to criteria on a 22 item, multi-task battery which evaluated visual, psychomotor, cognitive, and dual-task performance.

Two drug testing weeks were separated by one week of no drug administration. Daily doses of the drug or the placebo were given at 0700-0800, 1600-1700 and 2300-2400 for 5 days. Performance was tested at the same time of day for each subject on days 4 and 5 of each test week, and 3 days after drug intake ceased (day 8). A subgroup (N = 12) was also tested on day 2 of each drug week. Blood samples were obtained on days 3, 5 and 8 of each drug week. After the study, subjects received a second medical examination and debriefing interviews were conducted.

The drug regimen produced the expected mean level of inhibition in plasma cholinesterase. However, large individual differences in inhibition (range = -21.7% to +8.3%) were observed. No evidence of adverse health effects were associated with participation, or found in daily vital sign data. Measures of subjective state and daily work and life activities failed to distinguish between conditions.

On day 2 of drug intake, performance was worse on the visual probability monitoring task under pyridostigmine; no other effects were significant. The effects of chronic intake were evaluated using day 4 and 5 test data. Performance under pyridostigmine improved significantly on tests of depth perception, visual contrast sensitivity at 3 c/d, and hand steadiness. However, under the drug, decrements were found in dual task performance.

For example, when an attention task and an information processing task were performed simultaneously, greater performance decrements in information processing were found under the drug condition ($F = 5.39$, $p = .03$). Similarly, when a visual tracking task was performed simultaneously with a memory search task, there was a strong trend for the memory task to be more disrupted under pyridostigmine than under placebo conditions ($F = 3.15$, $p = 09$). This suggests that pyridostigmine may have a negative influence on the reserve capacity used by an individual when performing tasks requiring rapid attention sharing.

Regression analyses were performed to determine the performance consequences of individual differences in cholinesterase inhibition. As inhibition increased, performance on tests of visual acuity decreased, and depth perception improved. Finally, the greater the inhibition, the greater the increase in oral temperature.

These results suggest that pyridostigmine in the doses used is well tolerated by healthy young men. Although few decrements were observed, they were found in functions of particular importance to military operations. Further research to replicate and clarify these findings should be conducted.

I. INTRODUCTION

Pyridostigmine is a reversible anticholinesterase inhibitor long used in medical treatment of the neuromuscular disorder myasthenia gravis. Due to the site and the reversible nature of its action, the drug also has potential for use as a pretreatment medication to aid USAF personnel survival in the event of a chemical attack. Prior to use of the drug in this context, however, two major factors need to be evaluated. First, whether the drug presents any risk to the health of normal individuals, and second, whether the drug has any adverse effect on human functions important in USAF pilot operations.

Data are available concerning the health risks associated with pyridostigmine intake in both clinical and normal populations. Health risk is a function of the drug dose administered. Very high doses of pyridostigmine can result in death. Thus, when administering the drug to patients, the typical medical procedure is to gradually increase the drug dose to a point where clinical symptoms are controlled and drug side effects are minimal. Clinical reports indicate that relatively high daily oral doses of pyridostigmine, in excess of 600 mg/day, are tolerated well in patient populations.

Medical reports, however, only provide information on drug effects in patient populations; they are not directly relevant to determination of potential adverse health effects in normal individuals. Fortunately, recent NATO studies (Gall, 1981) have evaluated the effects of pyridostigmine in nonclinical populations. In these studies, hundreds of volunteers received 30 mg oral doses of the drug three times per day; some received the drug for more than 4 weeks. This drug regimen was reported to be well tolerated in the normal population evaluated, with little noticeable effect on daily life or work activities. Minor gastric upset and flatulence were the only drug side effects observed, and these were limited to a few subjects.

Although the health risks associated with this particular drug regimen were considered to be minimal, the consequences of drug intake on human functions of specific importance to aircraft pilot operations needed to be evaluated. Consequently, this research program had two major objectives. The first was to evaluate a preliminary test battery composed of a variety of tasks and measures related to pilot performance and/or drug action. The second objective was to use this battery in a controlled laboratory study of the effects of the selected drug regimen on the performance, physiology and subjective state of 24 healthy, young men.

II. METHODS

A. Subject Recruitment and Screening

Subjects were recruited by means of advertisements posted at local colleges and universities, and from our existing subject pool. Volunteers were asked to call MRI for more information about the research program. When a potential subject called, the purpose, procedures, risks and benefits were fully and accurately explained. If the subject agreed to participate, preliminary screening information was obtained. In general, participants had to be males in good health between the ages of 21 and 35 years, with normal vision (corrected) and hearing, and currently not taking any medication or using illicit drugs. They had to also agree not to use alcohol or drugs during the drug administration and testing phases of the program. Specific exclusion criteria included evidence of any of the following conditions: asthma, broncho-constrictive disease, dysrhythmias, prostatitis, urinary obstructions, ulcers, GI obstructions, seizure disorders, psychiatric problems, and recent acute illness.

Volunteers who met all preliminary screening criteria were scheduled for an intake interview at MRI. During this interview, the principal investigator again explained the purposes, procedures, risks and benefits of the program and answered any questions the volunteer had. The subject

signed the approved statement of informed consent and received a copy of his signed statement. A urine sample was obtained for analysis of licit and illicit drugs, a blood sample was obtained for determination of the subject's baseline level of plasma cholinesterase, and an appointment for a complete medical examination was made with the project physicians. If the results of the medical examination and the blood and urine assays met program requirements, the subject was scheduled for the remaining sessions in the program.

Screening interviews were conducted with 76 men. Of these, 33 met the initial screening criteria and volunteered to participate. Eight subjects were discontinued from the program before beginning the drug regimen: three because they failed to meet medical criteria; three because work or school schedule changes conflicted with the program schedule; one because he failed to meet training criteria on the test battery; and one because of family problems.

One additional subject was discontinued after beginning the first drug regimen. This subject reported suspected drug side effects. The actions taken by project staff in regard to this subject are documented in Appendix A. It was the opinion of the staff and project physician, based on blood analyses and other factors, that the reported symptoms were not directly drug-related, but that participation for this subject should be discontinued.

The remaining 24 volunteers completed all study requirements. Subjects were paid $3.50/hr for each hour of training and testing, and $5.00 for each pill ingested. Each subject who completed the study received a total of approximately $250.00 for the 40 to 50 hr of participation involved.

B. Experimental Design and Test Protocol

A double-blind, crossover experimental design was selected to take advantage of the statistical power provided by using each subject as his own control. The design and testing sequence are presented in summary form in Figure 1.

As shown in Figure 1, the initial week of participation involved learning to perform the task battery and becoming familiar with the procedures and forms that would be used during the drug administration phases of the program. On the final day of training, a blood sample was drawn to provide a second baseline measure of plasma cholinesterase. Training and familiarization activities required approximately 9 to 10 hr of subject participation.

Half the subjects (N = 12) were assigned at random to the drug administration sequence, pyridostigmine followed by placebo. The remaining subjects (N = 12) participated in the reverse sequence. There was 1 week without drug administration between the two drug regimens. All subjects followed the same schedule during each drug administration sequence. Pills (30 mg) were administered three times a day (morning, afternoon, and night) for a total of 90 mg/day for 5 consecutive days (Monday through Friday). During this time, vital signs were recorded every morning before the pill was ingested, and various subjective scales were completed at each pill administration. Blood samples were obtained at the same time of day on Wednesday, Friday, and the following Monday (days 3 and 5 of the drug regimen, and 3 days after drug ingestion ceased).

Performance testing sessions were conducted at the same time of day for each subject on Thursday, Friday, and the following Monday (days 4 and 5 of the drug regimen, and 3 days after pill intake was discontinued).

EXPERIMENTAL DESIGN

	Pill No. 1	Pill No. 2
Group 1 (N = 12)	pyridostigmine	placebo
Group 2 (N = 12)	placebo	pyridostigmine

TESTING SEQUENCE

Day		Week	Scheduled Activities (Medical Examination, Urine Screen, Blood Sample)		
1	M	1	Training		
2	T		Training		
3	W		Training		
4	R		Training		
5	F			Blood Sample	
6	S				
7	S				
8	M	2	Pill No. 1 (3 times each day)		
9	T		Pill No. 1		(Test Session, N = 12)
10	W		Pill No. 1	Blood Sample	
11	R		Pill No. 1		Test Session
12	F		Pill No. 1	Blood Sample	Test Session
13	S				
14	S				
15	M	3		Blood Sample	Test Session
16	T				
17	W				
18	R				
19	F				
20	S				
21	S				
22	M	4	Pill No. 2 (3 times each day)		Urine Screen
23	T		Pill No. 2		(Test Session, N = 12)
24	W		Pill No. 2	Blood Sample	
25	R		Pill No. 2		Test Session
26	F		Pill No. 2	Blood Sample	Test Session
27	S				
28	S				
29	M	5		Blood Sample	Test Session

(Post Participation Medical Examination)

Figure 1 - Summary of Experimental Design and Testing Sequence

In addition, half of the subjects from each drug administration sequence (drug-placebo, placebo-drug) had an extra performance testing session on Tuesday, the second day of pill administration. The purpose of this session was to test for early drug effects on performance. A urine sample was obtained on the morning of day 1 of the second drug sequence for screening of licit and illicit drugs. After each subject completed all program requirements, he was scheduled for a post-experimental medical examination with the project physicians.

The following procedures were used to maintain the integrity of the double-blind, and to protect the health of the subjects. The COTR provided MRI with a set of labeled packets (e.g., Subject 1, Drug 1; Subject 1, Drug 2), as well as bulk spares of each drug type. The COTR also provided a key to the double-blind code directly to the project physician, and to the office of the MRI Vice President for Chemical and Biological Sciences. As the pills were used, additional pills were dispensed to project staff from the Vice President's office. This procedure protected the integrity of the double-blind since project staff only knew that they were administering Drug 1 or Drug 2 to a particular subject. It also protected the safety of the subject since the project physician could examine his key and quickly determine if any reported effects were or were not likely to be drug related.

C. Task Selection and Implementation of Test Battery

Table 1 summarizes the battery of tasks and measures used in this program to evaluate the effects of pyridostigmine on functions relevant to pilot performance and/or drug action. Each battery item is described in detail in the next section of this report.

Individual tasks and measures were selected for inclusion in the battery through the coordinated efforts of MRI staff and AFAMRL/HEG and AFAMRL/HET personnel. A primary goal underlying the selection process was to ensure that a broad spectrum of performance capabilities would be assessed.

TABLE 1

TASK BATTERY SUMMARY TABLE

VARIABLE	ASSESSMENT METHOD
Physiological Measures	
Blood Pressure	Auscultation
Oral Temperature	Oral Thermometer
Pulse Rate	Palpation
Cholinesterase	Dietz modification, Ellman procedure
Pyridostigmine	GC-Mass Spectrometer
Visual Function	
Spatial Resolution	Contrast Sensitivity Task
Neural Transit Time	Steady State VER Task
Visual Acuity	Snellen Eye Chart
Depth Perception	Biopter Test
Psychomotor Function	
Eye-Hand Coordination	PPEB Tracking Task (single axis)
Coordination	Two-Hand Coordinator Task
Precision	Stabilimeter Task
Speed	Simple Reaction Time Task
Strength	Grip Strength Task
Perceived Exertion	Exertion Scale Rating Task
Central Processing	
Internal Timing	Interval Production Task
Memory - Span	Digit Span Task
- Processing Time	Sternberg Memory Task (set sizes 3, 4 and 6)
Attention - Monitoring	MTPB 3 Meter Monitoring Task
- Interference	PPEB Stroop Color/Word Task
- Perseveration	Reverse Tapping Task
Information Processing - Symbolic	Two Digit Addition Task
Decision Making	
- Integrated/complex	Baddely Grammatical Reasoning Task
- Choice	PPEB Forced Choice Reaction Time Task
Simultaneous Central Processing	
Eye-Hand Coordination with Memory Processing Time	PPEB Tracking Task (primary) with Sternberg Memory Task (set size 6) (secondary)
Attention with Information Processing (Symbolic)	PPEB Stroop Color Task (primary) with Two-Digit Addition Task (secondary)
Attention with Information Processing (Spatial)	MTPB 3 Meter Monitoring Task (primary) with Target Identification Task (secondary)
Subjective Effects	
Symptom Checklist	General Response Questionnaire
Fatigue	SAM Fatigue Scale
	MARI Fatigue Scale
Workload	SAM Workload Scale
	Subjective Workload Assessment Technique (SWAT)
Depression	Depression Adjective Check List (DACL)

Thus, the battery was structured within a theoretical orientation which provided measures of basic physiological and psychophysiological indices, critical visual and perceptual functions, psychomotor performance (understood as those skills involving neuromuscular control, precision and strength), and cognitive and other central processing functions. In addition, specific tasks in each of the above categories were selected on a theoretical basis to be performed together as simultaneous tasks. Multiple task performance was included in order to assess whether the drug had any unique impact under conditions where the workload on a subject was increased and he was required to divide attention and manage resources in order to perform two tasks simultaneously.

Subjective effects measures were also included in the battery. These measures were designed to evaluate whether or not the individuals: (a) were aware of any drug-related symptoms not apparent in the performance or physiological measures; and (b) perceived any differences in the subjective workload required to maintain performance. Finally, chemical measures were included to validate that other medication or illicit drugs were not exerting an unanticipated influence on performance, and to track the effects of pyridostigmine on individual cholinesterase inhibition.

Within the time and funding constraints of the contract, MRI performed major hardware construction and computer programming activities in implementing the task battery. The USAF supplied MRI with certain items of equipment and computer software to aid in implementation of the task battery. Major items of equipment included a Nicolet CS 2000 Contrast Sensitivity Testing System to collect contrast sensitivity data, and the equipment and computer software to present a multiple task performance battery developed by a previous contractor. This computerized performance battery was named the Psychomotor Performance Evaluation Battery (PPEB).

Project staff modified the PPEB software for compatibility with the current battery, reconstructed the response devices, and developed training procedures and testing criteria for the various subtasks used. MRI also

constructed a duplicate performance testing setup in order to be able to train and test multiple subjects in the timely and efficient fashion required. The specific PPEB tasks used were:

The Sternberg Memory Task (set size 3, 4 and 6)
Single Axis Tracking Task (horizontal)
Sternberg Memory Task (set size 6) with Tracking
The Stroop Color/Word Test
The Forced-Choice Reaction Time Test
The Multiple Task Performance Battery (MTPB):
- Probability Monitoring
- Warning Light Monitoring
- Blinking Light Monitoring
- Target Identification

In implementing the task battery, project staff developed the protocols required to train and test subjects on each battery task, and evolved a "station testing concept" that allowed us to train and test multiple subjects simultaneously in an efficient fashion. Project staff also set up the apparatus and constructed the electronic logic/computer software configurations required to present and collect data for the following specific tasks:

Steady State VER Task
Two-Hand Coordination Task
Simple Reaction Time Task
Interval Production Task
Reverse Tapping Task
Two-Digit Addition Task
Stabilimeter Task
Grip Strength Task
Depth Perception Task
Visual Acuity Task
Grammatical Reasoning Task

D. Procedures

1. Pill administration, vital signs, and subjective effects: The experimental design required that pills be administered three times per day: between 0700 and 0800 hr; between 1500 and 1600 hr; and between 2300 and 2400 hr. Prior to administering the morning pill, the experimenter measured the subject's: (a) temperature using a calibrated oral thermometer;

(b) pulse rate over the left radial artery; and (c) blood pressure by auscultation, using the disappearance of Korotkov sounds as the criterion for determining diastolic pressure. At this time, the General Response Questionnaire, an instrument developed by project staff to measure the subjective effects and symptoms associated with ingestion of pyridostigmine, was administered, as were the SAM fatigue and workload scales and the Multiple Adjective Rating Index (MARI), an alternative instrument developed by MRI for the measurement of fatigue.

The afternoon pill administration included the MARI and SAM scales and the Depression Adjective Check List (DACL). Equivalent forms of the DACL were given in counterbalanced order. At the night administration of the pill, the SAM and MARI scales were given again. A subjective measure of workload, the SWAT, was also obtained at the end of each performance test session.

The original protocol called for all doses to be administered by MRI personnel. This requirement markedly reduced the pool of available subjects since many potential subjects had class or work schedules which conflicted with one of the pill times. After consultation with the COTR, it was agreed that subjects could take one dose per day without supervision. This procedure was implemented only when absolutely necessary. The subject was given a vial containing one pill, together with the forms to be filled out at that pill administration. After completing the forms and taking the pill, the subject called an assigned project staff member to verify his compliance. If no call was received within 15 min after scheduled dosing time, the experimenter contacted the subject to remind him, and to verify compliance. This procedure protected against subjects accidentally forgetting to take the pill at the correct time. Fourteen subjects participated under this protocol. Subsequent examination of the data revealed no differences in cholinesterase inhibition between these subjects and those who followed the original protocol.

2. Biochemical measures

a. Serum cholinesterase: Serum cholinesterase hydrolyzes acetylcholine and certain other esters. The enzyme is synthesized in the liver and is present in high concentration in blood plasma. The Dietz modification of the Ellman procedure utilizes propionylthiocholine iodide as substrate and measures the reaction of the thiocholine formed on 5,5-dithiobis(2-nitrobenzoic acid). The yellow-colored end product is directly proportional to the cholinesterase activity in serum and is measured at 405 nm. Gilford Diagnostics reagents were modified for use on the Baker CentrifiChem 500 analyzer.

b. Pyridostigmine: The assay for pyridostigmine presented considerable difficulty. Two methods described in the literature (Blanchard, 1981; Chan et al., 1976; Ellin et al., 1982; Yakatan and Tien, 1979) were evaluated for possible use in the determination of pyridostigmine bromide levels in serum. Neither met our quality assurance requirements for sensitivity and reproducibility.

Several workers (Blanchard, 1981; Ellin et al., 1982; Yakatan and Tien, 1979) have cited procedures for the determination of pyridostigmine in blood serum by ion-pair reverse phase high performance liquid chromatography. Sensitivity for the detection of the drug was reported to be 20 to 40 ng/mL of serum. A number of problems were encountered at MRI when attempting to reproduce these procedures. Detection limits, determined using standard solutions, were found to be approximately 50 ng/injection; however, the detection limit for the drug in the serum matrix was considerably higher (150 ng/mL) due to the presence of coeluting endogenous serum components. Enhancement of selectivity was attempted by the use of a number of octadecyl analytical columns, as well as several alkyl sulfonate ion pairing reagents. Cleanup procedures included the use of ODS extraction columns and classical liquid-liquid extraction techniques. It was determined that these HPLC procedures were not selective enough to allow detection at the desired levels.

The gas chromatographic procedure of Chan et al. (1976) was also evaluated. This procedure is based on the quantitative dequaternization of the pyridostigmine salt in the inlet of the gas chromatograph, followed by separation on a packed column, and thermionic specific detection. Dequaternization was not found to be reproducible in work performed at MRI, in spite of efforts to provide extended residence times and catalytic surfaces in a superheated inlet. Additional problems encountered with the Chan procedure included column absorption and the inherent problem of matrix interferences, which is lessened but not obviated by the use of thermionic specific detection. It was concluded that this procedure was neither reproducible, stable, nor specific enough for use in the routine assay of pyridostigmine bromide in a large number of samples.

Considerable effort was then expended for methods development. Despite the fact that much of this effort was not charged to the project, the costs of methods development exceeded the budget for pyridostigmine assays. At this point, we have developed a highly sensitive, reproducible assay method. This is a major accomplishment, since the adequacy of assays has recently become an issue of concern to scientists working in the area.

The method developed for this program uses the sample cleanup procedure of Chan et al. (1976) followed by combined fused silica capillary gas chromatography/selected ion monitoring mass spectrometry. Neostigmine bromide is used as an internal standard.

Gas chromatography of the bromide salt of the iodine-glycine complex of pyridostigmine requires that quantitative thermal dequaternization occurs in the inlet of the instrument. Chan et al. (1976) reported that injection of both of these species into a heated inlet yielded a peak with a retention time identical to that obtained upon injection of the dimethyl carbamate ester of 3-hydroxypyridine. The identity of the dequaternization

product as the dimethyl carbamate ester of 3-hydroxypyridine has been confirmed from mass spectral data obtained at MRI. The capillary inlet splitter to be used, operated in the splitless (Grob) mode, is well-suited for this process.

Standard curves, without the use of an internal standard, have been validated over the range of 40 to 170 ng pyridostigmine bromide per milliliter of serum. These curves, based on measurement of the molecular ion of the dequaternization product (m/z 166) and the most abundant ion in the spectrum (m/z 78), were linear with correlation coefficients of 0.993 and 0.996, respectively. The use of an internal standard should yield even better correlations. In addition, slight modifications of the extraction procedures and detection parameters should allow accurate quantitation of pyridostigmine bromide at levels less than 20 ng/mL of serum. A limited study performed at MRI indicates that recovery is essentially quantitative using the cleanup procedure of Chan et al.

3. Performance measures: The performance test battery was organized around the concept of "work stations," with specific tests being conducted at each station. Table 2 identifies the stations and presents the tests conducted at each station. Performance data were collected on days 4, 5 and 8 of each pill regimen. Half the subjects had additional performance assessments to examine early drug effects on day 2. For any subject, all performance assessments were conducted at the same time of day.

a. Chamber station: All tests which required physiological recording or the use of the PDP 11/23 computer were conducted in an electrically shielded, sound attenuated chamber. The chamber was monitored by video and audio communication equipment located in an adjacent control room. The following tasks were performed at this station:

(1) Steady State Visual Evoked Response (SSVER): The SSVER was collected in order to provide a measure of neural transit time.

TABLE 2

TASKS AND MEASURES OBTAINED AT EACH WORK STATION

Monitoring Station

- Blood pressure
- Oral temperature
- Pulse rate
- Physical symptom checklist
- Blood draw
- Urine sample
- Subjective measures (MARI, SAM94, DACL)

All drugs were administered from this station, and subjects were paid for their participation here.

Chamber Station

- Steady state VER (mid and high frequency ranges)
- Simple reaction time task
- Reverse tapping task
- Interval production task
- Digit span task

Performance Station

- Grip strength/perceived exertion scale
- Steadiness task
- Two-hand coordination task
- Visual acuity
- Contrast sensitivity task
- Depth perception task
- Grammatical reasoning task

Apple Station

- Three-meter monitoring task
- Sternberg memory search task
- Stroop color/word task
- Two-digit addition task
- Forced-choice reaction time task
- Single axis tracking task
- Combined tracking and Sternberg tasks
- Combined Stroop and addition tasks
- Combined monitoring and target identification tasks
- SWAT

A gold cup electrode with EEG creme as the contact medium was attached to a cleaned and abraded site (Cz) and referenced to linked mastoids. The subject was seated in the chamber directly facing the SSVER stimulus apparatus, and 80 cm distant from it. The stimulus apparatus consisted of two fluorescent tubes mounted horizontally, with a fixation point located equidistant between the lights. The lights were modulated at maximum intensity through a D/A converter using either of two combined waveforms (a mid-range of 25, 32 and 38 Hz, and a high range of 46, 51 and 55 Hz). All combined waveforms were constructed using sine waves of equal amplitude.

All training and test trials were conducted in complete darkness. The identical protocol was followed for each trial. One of the two selected sets of combined frequencies was activated, and the subject instructed to begin fixating on the target between the lights. After 30 sec of fixation, data collection began. A 15-sec epoch of simultaneous EEG activity and fluorescent light modulation (picked up at the source by a photo-transducer) was collected at a sample rate of 256 Hz. During each test session, SSVER data were collected for two, 15-sec epochs to the mid-range frequencies and two, 15-sec epochs to the high range frequencies. These data were stored on disk for off-line FFT analysis.

(2) Simple reaction time: Apparatus included the PDP 11/23, a stimulus light, and a microswitch for recording the response. The subject was instructed to hold the index finger of his dominant hand over the switch, and to depress it as quickly as possible when a red light appeared in the panel in front of him. Twenty stimuli were presented at a random IS1 between 1,500 and 3,000 msec. Training criteria were considered to be met when reaction time averaged 250 msec or less, and the standard deviation did not exceed 20% of the mean reaction time. During each test session, subjects performed one practice trial followed by one test trial. Performance was evaluated using the mean and standard deviation of the test trial.

(3) Reverse tapping task: This task was presented and data collected using the capabilities of the PDP 11/23. Apparatus included

a tone generator for stimulus presentation and a microswitch for recording responses. Stimuli consisted of the presentation of a random series of 60 single and double tones. The subject's task was to press the microswitch twice in response to each single tone, and once in response to each double tone. Training began at an interstimulus interval (ISI) of 1,500 msec. When the subject was able to respond correctly 90% of the time, ISI was reduced to 1,200 msec. As the subject learned the task, ISI was reduced in 100-msec steps until he could maintain 90% accuracy at an ISI of 900 msec for two consecutive trials. During actual testing, subjects first performed one practice trial and then performed one test trial, both at the 900-msec rate. Percent correct on the test trial was the performance measure.

(4) Interval production task: This task provides a measure of variability of internal timing. The subject was instructed to depress a microswitch at a steady rate of two to three times per second. The PDP 11/23 computer collected data on the rate and variability of task performance over a 2-min period. Data collection began 20 sec after the subject initiated the task. Subjects were trained to stable performance (criterion: IPT score < 20.0 for two sequential 2-min trials). During each test session, subjects first performed a 30-sec practice trial, followed immediately by a 2-min test trial. Task performance was evaluated using the IPT score as a measure of variability.

(5) Digit span task: The digit span subtest of the Wechsler Adult Intelligence Scale was used to measure the span of memory, both forward and backward. Using previously determined random orders of digits, the experimenter read numbers over the intercom at a rate of one number per second; the subject then repeated the numbers, either in the order given or in the reverse order, depending on the instructions. Digits forward was tested over the range 4 to 9 digits, while digits backward was assessed from 3 to 8 digits. Testing continued until the subject failed a given set size twice in a row. The largest set of digits recalled correctly was used as the measure of memory span. During training, the subject was familiarized with the task by performing both digits forward and digits backward three times. During test sessions, subjects performed the task once in each session.

b. Performance station: Equipment for all seven tasks to be performed at this work station was located around the perimeter of a large room, and the subject and experimenter moved from task to task.

(1) Grip strength and scale of perceived exertion: A Lafayette Hand Dynamometer No. 76618 (range, 0 to 100 kg) was employed. A chart showing the Perceived Exertion Scale was posted on the wall in front of the subject. The dynamometer was individually adjusted for the dominant hand of each subject, and the setting recorded. The same setting was used throughout the expermental period for that subject. The arm was held cocked at the elbow and parallel to the floor during assessments. Two trials were performed with the dominant hand, alternated with two trials using the non-dominant hand. At the end of each trial and before looking at the Dynamometer reading, the subject reported the level of effort exerted during that trial. The entire procedure was repeated twice during training for familiarization purposes. During the experimental period, the highest grip strength achieved in a session for both the dominant hand, and the nondominant hand, was entered into statistical analysis, as were the exertion ratings associated with those trials.

(2) Steadiness: A Lafayette Steadiness Tester No. 4605C was connected through electronic logic to an automatic timer. The subject's task was to hold a stylus in a hole without allowing it to touch the sides of the hole. Care was taken to assure that the subject maintained a standard hand and wrist position during training and during all test sessions. Subjects worked progressively from the third largest to the smallest diameter holes (0.25 to 0.078 in.), keeping the stylus inside each hole for 10 sec. The total number of seconds of contact between the stylus and the walls of the seven holes was the performance measure. During training, two trials were given to familiarize the subject with the procedure. During each test session, subjects performed the task once.

(3) Two-hand coordination task: This task was used to assess visual-motor coordination. On the Heinrich Model Two-Hand Coordination device, the target moved through an irregular circular pattern at variable

speed. The subject's task was to track the target by simultaneously using one hand to control the forward-backward movement of the tracking cursor, and the other to control its left-right movement. Training criterion was met when the subject could demonstrate 80% time on target for four consecutive 1-min trials. During training, the subject practiced in blocks of five trials each. Trial blocks were separated by practice on other tasks. During testing, the subject performed four consecutive trials; mean time on target was the performance measure.

(4) Visual acuity: Standard eye charts were used to assess static visual acuity. In order to provide sufficient charts for all test sessions, both Snellen and "illiterate" charts were used. Subjects stood 20 ft from the chart, which was kept covered at all times when not in actual use. Training consisted of two familiarization trial using the letter chart. Acuity was determined on test day 4 using the "illiterate" chart; on day 5 the Snellen letter chart was used; and on test day 8, acuity was determined using an upside down "illiterate" chart. The standard acuity value was used as the performance measure.

(5) Contrast sensitivity: The Nicolet CS 2000 Contrast Sensitivity Testing System was used to present static sinusoidal gratings of 0.5, 1.0, 3.0, 6.0, 11.4 and 22.8 cycles per degree (c/d). During training and testing sessons, subjects were seated 3 m from the viewing screen. All sessions were conducted under incandescent room illumination. The standard Von Bekesy tracking method was used to train subjects at each grating setting. If reasonable stability was not achieved at any particular setting, the "probe" technique was also used. Performance parameters (mean, standard deviation and sensitivity score) were automatically calculated by the CS 2000 and printed out after each trial. During test sessions, subjects performed the standard test programmed by the CS 2000. This consisted of the presentation of eight separate trials, each of which presented a single grating. The first two trials (0.5 and 6.0 c/d) were practice trials. The remaining six trials (0.5, 1.0, 3.0, 6.0, 11.4 and 22.8 c/d) were data collection trials.

(6) Depth perception: Lafayette Depth Perception Apparatus No. 1702 was used. The subject was seated 15 ft from the apparatus, which was at eye level. The task was to use strings to adjust two rods inside the apparatus so that they appeared to be at equal depth. During training, the subject was given two familiarization trial blocks, each consisting of four trials starting from different rod positions. Testing sessions also consisted of four trials, each starting from a different rod position. The deviation, in millimeters, and its direction were recorded for each trial, and the mean absolute deviation entered into statistical analysis.

(7) Grammatical reasoning test: Ten randomized versions of the 32-item Baddeley Grammatical Reasoning Test were prepared for the study and administered to subjects in random order. During the first training session, the experimenter explained the test, and demonstrated the solution to the first two problems using a form with items listed in order of increasing difficulty. The subject then completed the rest of the test. This was immediately scored, and any errors were reviewed with the subject to assure that he clearly understood the directions. Two subsequent presentations of the test with performance feedback occurred during the training period. During the test sessions this task was performed once. Both the time required to complete all items and the percent correct responses were recorded for analysis.

During protocol development, some subjects complained of after-images interfering with their ability to make accurate judgments. We contacted the manufacturer, and it was suggested that we remove the "Preview" feature of the Standard Test. This feature presents the subject with a 2-sec full-contrast "preview" of each grating to be tested. Consequently, this feature was not included in the Standard Test.

c. Apple station: There were two Apple stations; one consisting of the complete computer configuration and response board, and the second set up for all tasks except the single axis tracking, Sternberg, and 2-digit addition tasks. Both stations were used to train and test multiple subjects simultaneously on the tasks performed at this station. All but one

of the following tasks used the PPEB software. This software is also described in the Users Manual prepared by MacAulay-Brown, Inc., under USAF Contract No. F33615-80-C-0514.

(1) Three-meter monitoring task: One subtask of the PPEB is the Multiple Task Performance Battery (MTPB). The MTPB can present five separate tasks simultaneously or in various combinations. A low workload condition can be simulated by having the subject perform the following three tasks simultaneously.

(a) Probability monitoring: Four sets of six vertical bars each were displayed at the top of the computer display, with a moving dot under each set. The four dots moved through a regular, repetitive sequence (i.e., the dots moved under bars 1, 3, 5, 2, 4 and 6). At randomly selected intervals, the dot under one set would begin an abnormal sequence. The subject's task was to monitor the sets, and detect and correct the abnormal condition by depressing the appropriate button on his response panel. The subject had 2 min to respond, and depressing any button on the panel corresponding to these four sets of bars would correct the abnormal condition. The MTPB software provided measures of mean reaction time, correct detection, and false alarms.

(b) Warning light monitoring: In this subtask, a rectangle formed by two squares was presented on the screen. In the normal state a "G" was displayed in the top box. At random intervals, the "G" changed to an "R" in the bottom box. The subject had 5 sec to correct this abnormal condition by pushing a button on his panel. Mean reaction time, number correct, and false alarms were collected.

(c) Blinking light monitoring: A small shaded square alternated between two vertical boxes at a rate of 0.5 sec/alternation. At random, the alternation stopped but the square continued to blink at the 0.5 sec rate. The subject had 30 sec to correct this condition by pushing the appropriate button on the response panel. Mean reaction time, number correct, and false alarms were collected.

During training, the subject practiced the three-meter monitoring task for three 10-min trials. During test sessions, one 10-min trial was performed each session.

(2) Sternberg memory task: The PPEB software in conjunction with a speech synthesis system was used to present this task. Prior to testing, subjects memorized sets of three letters (A, H and J), four letters (A, H, J and Q), and six letters (A, H, J, Q, S and X). These letters were drawn from what was called the positive set. The negative set consisted of the letters B, C, E, F, G, I, L, R and Y. In performing the task, the subject was first told which set size was being tested, then he listened to the speech system randomly present letters from the positive and negative sets at a random ISI between 3.0 and 5.0 sec for a 2-min period. For each letter presented, he determined whether or not it was a member of the memorized set being tested and depressed the appropriate button on his response panel. Mean reaction time over all responses was calculated by the program and displayed at the end of the task.

During training, subjects practiced this task three times at each set size. During each test session, subjects performed the task once at each set size. Mean reaction time per set size was the performance measure obtained.

(3) Stroop color/word task: This task was presented using the PPEB software. On the video display screen, the subject was simultaneously presented with the color names "green," "blue," "red" and "yellow." Below each name was a Roman numeral (I-IV). These numerals corresponded to four response buttons on the subject's response panel. The color names were displayed randomly in a different color (e.g., the name "red" in the color blue). At the bottom of the screen in black and white, the subject saw either the stimulus "word" or "color" followed by a color name (e.g., word: red). If the stimulus was "word," as in the example, the subject searched the display for the word "red," and depressed the appropriate Roman numeral key on the response panel. If it said "color," he searched for the designated color and depressed the appropriate key. Immediately after the subject

responded, a new set of four color-word combinations was presented. The task duration was 2 min. Both accuracy and mean reaction time were automatically calculated. During training, subjects practiced the Stroop for a total of six 2-min trials. During testing, the Stroop was performed once each session.

(4) Two-digit addition task: This task is an externally paced, speeded addition task. Prerecorded audio tapes were prepared and presented in counterbalanced order. Each tape contained 15 random two-digit addition problems (e.g., 48 and 37) presented at 5-sec intervals. The subject's task was to mentally add the digits presented, and say his answer out loud before the next stimulus pair was presented. The task duration was 2 min. The number of correct additions and the number of missed response intervals were the measures obtained. During training, subjects practiced this task for three 2-min trials. During testing, the task was performed once each testing session.

(5) Forced-choice reaction time task: The PPEB software was used to present this 2-min task. Four boxes were presented on the screen in front of the seated subject. Four buttons on the response panel corresponded to these boxes. One box would fill at random, and the subject's task was to press the buttom on this panel that corresponded to the filled box. As soon as any button was depressed, the display would change and a new box would be seen as filled. Performance measures included mean reaction time and percent correct. Subjects performed this task four times during training, and once each test session.

(6) Single axis tracking: The PPEB task battery contains a subtask capable of presenting either a single or dual axis tracking task. No information was available on training or performance norms. Pilot tests of this task indicated that training time for dual axis tracking would be excessive. Additional pilot studies were conducted to determine appropriate parameter settings for single axis tracking, and the approximate number of training trials required to achieve stable performance. Based on the pilot study results, the single axis task was set for a maximum horizontal

deflection of 25 degrees. The seated subject manipulated a finger-controlled joystick to maintain a cursor on the Apple computer screen within a small square target moving horizontally at random across the screen. Movement of the joystick to the right or left caused the cursor on the screen to move proportionately in the opposite direction.

The PPEB program calculated RMS error every 0.1 sec for each standard 2-min tracking trial. If the joystick was not moved during a 2-min trial, the RMS error was 17.0 at the standard parameters. Training criterion for this task was set at an RMS error of 7.0 or less on two consecutive 2-min trials. Training was conducted in blocks of five trials interspersed with performance of other tasks. Subjects required up to 25 trials to achieve criterion. During each test session, subjects performed one practice trial and one test trial. The RMS error during the test trial was used as a measure of tracking ability.

(7) Single axis tracking task with Sternberg memory task: The motor performance task was designated as the primary task, and the memory task as the secondary task. Subjects performed both tasks simultaneously for a 2-min period. The Sternberg was performed at set size 6. Performance measures included the mean RMS error for the tracking task, and the mean reaction time and number correct for the Sternberg task. During training, subjects practiced performing each task alone and both tasks simultaneously. During testing, subjects performed the dual task once, immediately after performing each task separately.

(8) Stroop color/word task with two-digit addition: The Stroop task was designated as the primary task, and the two-digit addition task as the secondary task. During training, subjects performed each task separately, and then performed both tasks simultaneously for two trials. During testing, each task was performed separately, followed immediately by one trial of the combined task. Mean reaction time and percent correct were collected for the Stroop task, and percent correct was collected for the addition task.

(9) Three-meter monitoring with target identification task: The target identification task was presented as part of the MTPB. In the center of the display, the subject was presented for 5 sec with a target histogram consisting of six bars of varying lengths. The target was then erased, and followed at 15-sec intervals by two other histograms in random orientations. The subject used his response panel to indicate whether the first, second or neither of the following histograms matched the target. The subject was allowed 25 sec to respond. Percent correct was the performance measure obtained.

During training, subjects were first familiarized with the target identification task alone, and then performed the monitoring and target identification tasks simultaneously for two 10-min trials, with monitoring being designated as the primary task. During testing, the combined task was performed for one 10-min test trial each session.

III. RESULTS

The data were analyzed to answer the following major questions:

* Did ingestion of pyridostigmine produce the expected inhibition of cholinesterase?

* Could subjects or experimenters distinguish between pyridostigmine and placebo ingestion?

* What subjective effects, if any, were associated with chronic drug intake?

* Did pyridostigmine affect vital signs?

* Does a 1-day intake regimen of pyridostigmine result in changes in performance?

* Does a 5-day intake regimen of pyridostigmine result in changes in performance?

* Are individual differences in cholinesterase inhibition reflected in performance scores?

Table 3 presents an overall summary of findings. This section of the report presents the details of the various analyses conducted.

A. Plasma Cholinesterase (ChE)

Plasma cholinesterase data were submitted to 2 x 2 x 3 (Order by Drug by Day) analysis of variance (ANOVA) with repeated measures on the last two factors. Ingestion of pyridostigmine resulted in lower levels of plasma cholinesterase ($\bar{x}$ = 5,428 IU/L) than did ingestion of placebo ($\bar{x}$ = 5,710 IU/L) ($F = 8.52$, df 2,22, $p < .01$). Figure 2 shows the significant Drug x Day interaction ($F = 3.63$, df 2,44, $p < .05$). Under placebo conditions, cholinesterase did not change significantly. When subjects ingested pyridostigmine, levels were significantly lower on Day 3 ($t = 3.10$, df 23, $p < .01$) and day 5 ($t = 2.37$, df 23, $p < .05$), than on Day 8 (approximately 64 hr after the last dose). Days 3 and 5 did not differ from one another ($t = 0.43$, df 23, $p < .20$).

Paired t-tests were used to test specific hypotheses about cholinesterase inhibition. Mean baseline cholinesterase levels were compared with mean levels during dosing. While no difference was found for placebo ($t = .42$, df 23, n.s.), pyridostigmine reduced cholinesterase levels from baseline ($t = 4.98$, df 23, $p < .001$). Day 8 was not different between placebo and pyridostigmine regimens ($t = .12$ df 23), nor did day 8 differ from baseline ($t = .68$, df 23).

TABLE 3

OVERALL SUMMARY OF SIGNIFICANT RESULTS

Variable	5-Day Drug Effect?	1-Day Drug Effect?	Affected by Degree of ChE Inhibition? Regression	Group Comparison ($p < .20$)
Visual Function				
Depth Perception	Yes (+)	No	Yes (+)	Yes (+)
Visual Acuity	No	No	Yes (-)	Yes (-)
Contrast Sensitivity (3 c/d)	Yes (+)	No	No	No
Psychomotor Function				
Hand Steadiness	Yes (+)	No	No	No
Grip Strength	Yes (?)	No	No	Yes (+)
Simple Reaction Time	No	No	No	Yes (-)
Central Processing (Single Tasks)				
Memory				
Digit Span Backwards	No	No	No	Yes (+)
Sternberg Memory Task	No	No	No	Yes (-)
Attention				
Probability Monitoring	No	Yes (-)	No	No
Blinking Light Monitoring	Yes (?)	No	No	Yes (-)
Stroop Color Word Test	No	No	No	Yes (-)
Information Processing				
Two-Digit Addition	No	No	No	No
Simultaneous Control Processing (Dual Tasks)				
3-meter monitoring (Primary Task)			No	No
Probability monitoring	Yes (?)	No	No	No
Blinking light monitoring	Yes (?)	No	No	No
Target Identification (Secondary	No	No	No	Yes (+)
Stroop color word (primary task) (% correct)	Yes (+)	No	No	No
Two-digit addition (secondary)	Yes (-)	No	No	No
Tracking (primary)	No	No	No	Yes (-)
Sternberg memory task (secondary)	No	No	No	Yes (+)

+ = Performance improved under pyridostigmine.
- = Performance declined under pyridostigmine.
? = Complex interaction effect.

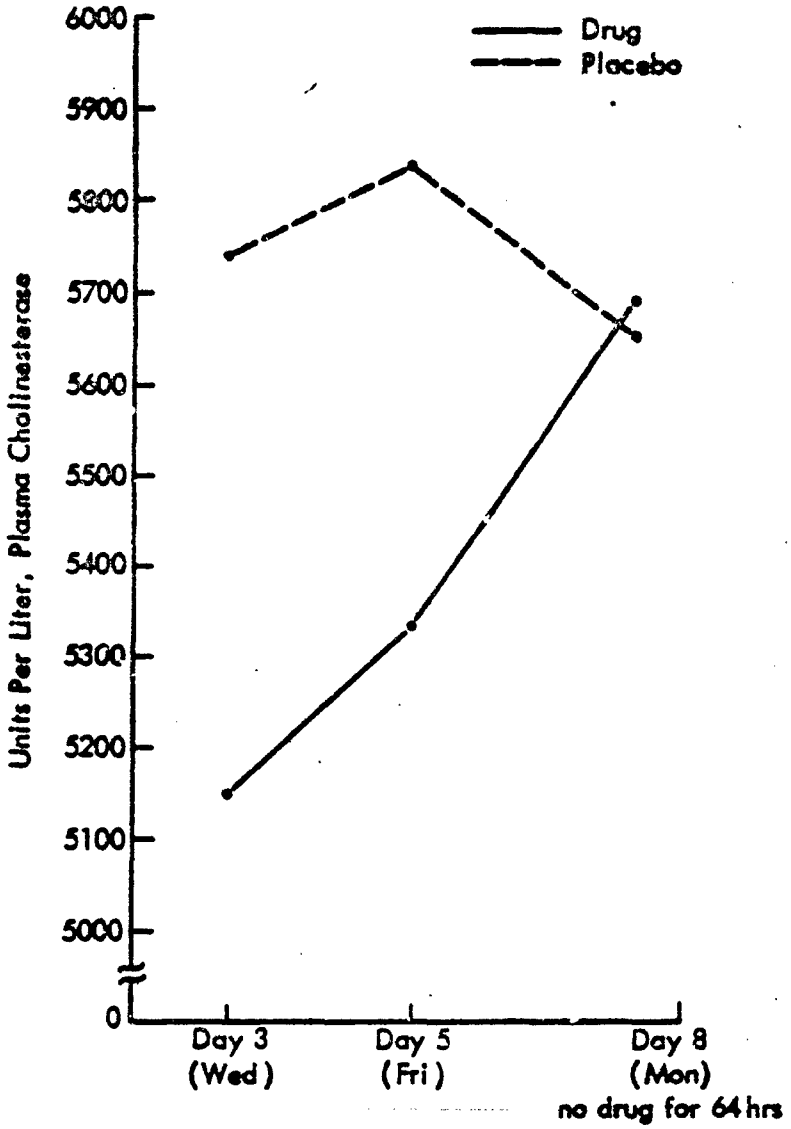

Figure 2 - Changes in Plasma Cholinesterase Level After Ingestion of Pyridostigmine and Placebo.

Pyridostigmine ingestion resulted in reduced levels of plasma cholinesterase, which returned to baseline when sampled 64 hr after drug intake ceased. The mean level of cholinesterase inhibition was 8.2%. This figure is lower than the inhibition levels reported in recent NATO studies (Call, 1981). The difference is probably due to our assays being conducted in plasma, and the NATO assays being conducted on red blood cells. The plasma results reported here are essentially equivalent to the NATO results, when the difference in assay material is taken into account.

A large degree of individual differences was observed in cholinesterase inhibition. Plasma cholinesterase inhibition under the identical conditions of drug intake ranged from 21.7% inhibition to 8.3% increase. Table 4 presents the data obtained and the percent inhibition for all 24 subjects over all blood sampling points in the study.

For clarification of these large individual differences we first conducted a complete examination of our procedures and protocols to determine if they could account for the differences observed. The biochemical assay procedures were reevaluated for reproducibility and sensitivity. Cumulative coefficient of variation (CV) % across assays was 3.5% for the high standard and 3.2% for the low standard; assay variability does not, therefore, explain the results obtained.

The COTR was contacted about the origin and age of the pills sent to MRI. The pills were a standard preparation freshly made for this study. We took pills at random from those designated Drug 1 and Drug 2, and assayed them. The drugs were as they should be. We rechecked our pill dispensing records with those in the Vice President's office. No problems were noted. Finally, since 14 subjects had taken one of the daily pills outside the direct observation of project staff, inhibition levels for these subjects were compared to inhibition levels for the remaining subjects. The groups did not differ.

TABLE 4

INDIVIDUAL DIFFERENCES IN PERCENT PLASMA CHOLINESTERASE INHIBITION UNDER CHRONIC ADMINISTRATION OF PYRIDOSTIGMINE VERSUS PLACEBO

(30 mg, 3 x day, 5 days)

	Units per Liter							% Inhibition	
	Pre-experimental Baseline (Mean of	Pyridostigmine			Placebo			from	from
Subject No.	2 Samples	Day 3	Day 5	Day 8	Day 3	Day 5	Day 8	Baseline	Placebo
1	6168	5514	5359	5648	7368	6388	5941	- 11.9	- 21.0
3	4618	4177	4157	3725	4335	5419	4037	- 9.8	- 14.6
4	7108	6410	6164	6761	5954	6633	6678	- 11.6	- 0.1
5	5411	4836	6315	5202	6129	5453	5271	+ 3.0	- 3.7
7	5682	5563	6739	6099	6443	5529	5765	+ 8.3	+ 2.8
8	4512	4224	3764	4366	4939	4582	4588	- 11.5	- 16.1
10	7872	7851	7254	8143	7716	7246	6396	- 4.1	+ 0.9
11	8608	7648	6898	8070	7829	9064	8264	- 15.5	- 13.9
12	4834	4458	4731	5619	5049	5062	4833	- 5.0	- 9.1
16	7004	7644	6728	7163	5863	7586	6975	+ 2.6	+ 6.9
19	7328	6011	6063	6298	6620	7120	7604	- 17.6	- 12.1
20	6666	5208	5287	6991	6033	6238	6667	- 21.3	- 14.5
21	4168	3176	3352	4567	3117	3775	4176	- 21.7	- 5.3
22	5924	5471	5186	5212	5604	5391	4914	- 10.1	- 3.1
23	4902	4570	4805	5045	5656	4939	4516	- 4.4	- 11.5
24	5242	4918	4754	4537	5939	5350	5012	- 7.7	- 14.3
25	6527	5475	6565	6310	6406	6962	6997	- 8.4	- 9.9
26	4761	5158	5459	4565	5021	4746	4740	+ 7.3	+ 2.6
27	5220	4734	4339	5501	4907	5642	5656	- 13.1	- 14.0
31	5153	4870	4726	5857	3282	5670	5689	- 6.9	+ 7.2
32	5546	4568	5255	5652	5189	6038	5650	- 11.4	- 12.5
33	3470	3140	2923	2910	3806	3612	3519	- 12.6	- 18.3
34	5366	5154	5278	5105	5536	5249	4599	- 2.8	- 3.3
35	6348	5735	5644	7238	6187	6333	6913	- 10.4	- 9.1
Mean	5674	5271	5323	5691	5622	5834	5675	- 8.2	- 7.8
S.D.	1234	1214	1139	1294	1219	1223	1180	7.9	8.0

B. Vital Signs

Systolic and diastolic blood pressure, pulse rate and oral temperature measures were obtained. Table 5 summarizes the data. No significant differences directly attributable to pyridostigmine were found.

C. Subjective Measures

Subjective measures included judgments by both experimenters and subjects of whether pyridostigmine or placebo had been ingested, a symptom checklist, the Depression Adjective Checklist, the SAM Fatigue and Workload Scales, and the Subjective Workload Assessment Technique.

1. Double-blind rating: Experimenters were not able to judge at better than chance levels (Fisher's Exact Test) whether subjects received pyridostigmine or placebo. During week 1 of the drug regimen, the subjects' ratings were also no better than chance. At the end of week 2, however, no subject taking placebo judged it to be pyridostigmine, so that ratings were significantly better than chance ($p < .05$, Fisher's Exact Test).

2. General response questionnaire: Subjects reported more symptoms ($\bar{x} = 98$) while taking pyridostigmine than while taking placebo ($\bar{x} = 80$). The difference approached significance ($F = 344$; df 1,22, $p < .10$), as did the interaction between order and drug ($F = 344$; df 1,22, $p < .10$). Nine of the 12 subjects who took pyridostigmine prior to placebo had higher symptom scores for pyridostigmine ($\bar{x} = 89$ versus 51.5), while only 5 of the 12 subjects who took placebo first showed such a pattern. The trends observed appear therefore to reflect, at least in part, a tendency toward "over-reporting" during the first week. Analysis of symptom scores by day yielded the same pattern. Because of the large number of variables and the relatively small number of subjects, factor analysis of the checklist was inappropriate. The instrument does, however, show promise and should be more fully developed for future work.

TABLE 5

SUMMARY OF VITAL SIGN MEASURES

Variable	Pyridostigmine	Placebo	Drug Main Effect			
			F	MS_{error}	df	p
Systolic Blood Pressure	113.3	114.0	1.40	31.28	1,22	n.s.
Diastolic Blood Pressure	70.9	70.4	.57	36.39	1,22	n.s.
Oral Temperature	97.5	97.4	1.78	.42	1,22	n.s.
Pulse Rate	64.8	65.1	.15	49.62	1,22	n.s.

3. Other subjective measures: None of the other subjective measures resulted in significant effects which could be attributed to the drug.

4. Independent interviews of study volunteers: After all data collection was completed, the opportunity arose for the subjects to be interviewed independently about their experiences during the study. Dr. Frederick W. Hegge (WRAIR), of the Tri-Service Committee, requested permission to speak with the study volunteers. This request was cleared through the COTR and approved by the MRI Human Subjects Committee.

The interviews were voluntary, and no payment was involved. Group interviews with 11 subjects were conducted on October 20-21, 1983, by Dr. Hegge and Col. Tyner (WRAIR). MRI staff members were not present during these interviews. Dr. Hegge reported that the subjects perceived no specific symptoms associated with drug intake, and the majority could not distinguish between the drug week and the placebo week. Their daily work was not disrupted (except by the study procedures), and their other life activities continued as before.

D. Performance Measures

1. Visual function: Table 6 summarizes the results obtained for measures of visual function.

a. Visual acuity: No differences in visual acuity attributable to pyridostigmine were found. The apparent improvement in acuity over time, indicated by a significant Order x Drug interaction ($F = 3.63$, df 2,22, $p < .05$) in the absence of any main effect for drug ($F < 1$), is probably a result of not counterbalancing the three eye charts which were used.

b. Depth perception: Depth perception was better under pyridostigmine ($\bar{x} = 14.2$ mm deviation) than under placebo ($\bar{x} = 17.9$ mm deviation) ($F = 7.54$, df 1,22, $p < .05$). No other main effects or interactions were significant.

TABLE 6

SUMMARY OF VISUAL PERFORMANCE RESULTS

Variable	Day	Mean Performance Score		Drug Main Effect			
		Pyridostigmine	Placebo	F	MS_{error}	df	p
Visual Acuity (Snellen notation 20/X)	4	16.7	16.6				
	5	15.4	15.4				
	8	15.9	16.4				
	Across Days	16.0	16.1	.09	4.73	1,22	n.s.
Depth Perception (mm error)	4	15.7	18.8				
	5	13.2	17.1				
	8	13.8	17.9				
	Across Days	14.2	17.9	7.54	65.92	1,22	.05
Contrast Sensitivity (Sensitivity Index) Setting	0.5	39.6	40.7	.17	265.69	1,22	n.s.
	1.0	128.1	119.1	1.04	2,775.81	1,22	n.s.
	3.0	376.6	326.9	4.28	20,724.00	1,22	.05
	6.0	317.6	326.1	.07	36,860.30	1,22	n.s.
	11.4	188.7	187.4	< .01	20,135.90	1,22	n.s.
	22.8	57.9	60.5	.17	1,394.09	1,22	n.s.

c. Visual contrast sensitivity: Visual contrast sensitivity was measured at 0.5, 1.0, 3.0, 6.0, 11.4 and 22.8 cycles per degree. A significant Order by Drug interaction ($F = 5.20$, df 1.22, $p < .05$) indicating a practice effect, was found at 0.5 cycles per degree. At 3 cycles per degree, a main effect for drug was found; contrast sensitivity was better under pyridostigmine ($\bar{x} = 376.6$) than under placebo ($\bar{x} = 326.9$) ($F = 4.28$, df 1,22, $p = .05$). No other effects were significant. It should be noted that contrast sensitivity data were highly variable, both within and between subjects.

d. Steady state visual evoked response (SSVER): USAF data reduction and analysis procedures were to be used to convert SSVER data to appropriate measures of neural transit time. Unfortunately, these analysis procedures were dependent, in part, on a specific piece of equipment unavailable to us within the period of this contract. Programming of substitute computer software was also beyond the scope of the contract. Therefore, the following limited analyses were conducted.

FFT analysis of the visual stimulus data was conducted using the Laboratory Subroutine Package (DEC, RT-11, Version 4.0, FORTRAN IV, Version 2.5). Data were submitted to FFT analysis in 2-sec epochs, using a 50% redundant sliding window which produced 14 FFT outputs with a 0.5 Hz resolution across a band ranging from zero to 128 Hz. Inspection of the FFT values for the visual stimulus data revealed that the hardware constructed by MRI produced an extremely clean signal. FFT amplitudes at the stimulated frequencies were approximately 70 times larger than at sideband frequencies, and the phase values of the signal channel demonstrated standard deviations of less than one degree for the 14 samples obtained on any one trial.

Measures of neural transit time are derived from the phase differences calculated between simultaneous measures of SSVER activity and the visual stimuli. The underlying neurophysiological mechanism involved is the capacity of the brain to "follow" or become entrained through exposure to specific visual stimulus frequencies. We evaluated whether pyridostigmine exerted any apparent influence on EEG "following" activity. EEG data for all 24 subjects obtained during exposure to the combined set of

high frequencies on Day 5 of pyridostigmine administration were compared to similar data obtained on Day 5 of the placebo administration. Table 7 shows the average signal-to-noise ratio for each subject at each of the high range frequencies presented. Inspection of Table 7 suggests that pyridostigmine exerts no systematic influence on EEG entrainment activity.

Half of the subjects were tested in the sequence drug followed by placebo; the remaining subjects were tested in the reverse sequence. Statistical tests indicated that drug administration sequence also had no systematic influence on EEG entrainment activity.

It is apparent that the SSVER data collected are highly variable, both within and across subjects. For example, Subject No. 31 (Table 7), shows very large S/N ratios under placebo conditions, and almost no evidence of entrainment under pyridostigmine administration. However, subjects 8, 22 and 33 show the reverse pattern. Since Table 7 only presents the data obtained from the Day 5 test sessions, it was of interest to further examine SSVER data obtained on the other test days, and also to evaluate SSVER activity in response to stimulation by the mid-range frequencies. FFT amplitude and frequency data for the stimulated frequencies were evaluated. EEG data obtained during the baseline training period and all test sessions, for all subjects, were evaluated and categorized using a 5-point evaluation scale. This scale ranged from (1) not distinguishable from background to (5) greater than twice the background activity level. A large degree of variability, both within subjects and across groups and conditions, was apparent.

It is not clear why SSVER activity should show such variability under identical conditions of stimulation. It should be noted, however, that the results reported here are not unique. Recent reports by USAF laboratories also indicate variation in the degree of following observed, and further, they state that such variation has little impact on the derivation of neural transit time measures from SSVER data. These preliminary analyses, however, do indicate the need for future standardized procedures in regard to stimulus exposure duration, test frequency selection, modulation amplitude, data collection epoch, and analysis algorithms. They also suggest that future studies

TABLE 7

EFFECTS OF CHRONIC ADMINISTRATION OF PYRIDOSTIGMINE VERSUS PLACEBO ON SSVER ACTIVITY* (SIGNAL TO NOISE RATIO)

Subject No.	Placebo Frequency			Pyridostigmine Frequency		
	46	51	55	46	51	55
1	-1.56	-1.97	.79	-2.08	-1.92	-.70
3	2.42	.52	-3.12	1.43	-.39	-.42
4	-2.84	-1.46	-.28	-.86	1.62	-.16
5	10.34	10.17	9.43	7.0	7.76	8.44
7	-.78	.20	-2.81	-3.92	-.67	-2.84
8	4.66	3.75	-1.26	13.73	14.95	14.07
10	-1.66	-3.43	-1.24	-4.0	-4.92	-1.68
11	-3.32	.85	-1.76	-2.9	1.91	3.22
12	-2.01	3.72	1.88	3.32	2.56	3.18
16	8.47	6.12	6.71	-.93	.90	-.30
19	1.44	3.66	2.52	-2.46	1.0	-2.81
20	-1.08	.01	-3.46	-4.26	.48	-1.50
21	2.0	-1.48	.30	4.38	5.92	8.0
22	1.75	.92	.58	7.68	8.98	8.2
23	-2.87	-.18	-1.15	.44	1.29	-1.42
24	1.02	-.36	-.83	5.72	4.12	3.36
25	.14	3.12	.82	4.48	8.44	4.97
26	-2.94	-1.84	-.38	-2.0	-1.05	-1.96
27	-3.50	-1.46	-2.08	.22	-2.24	-1.16
31	15.75	22.25	22.23	-3.93	-.56	-.78
32	10.08	11.44	10.84	9.08	8.86	9.71
33	-.55	-2.2	-2.56	12.25	10.11	10.84
34	-1.42	-2.16	-3.07	-2.13	-1.70	-.56
35	-2.42	-1.60	-1.8	-3.56	-3.27	-1.38

* $S/N = 10 \text{ LOG } \frac{p}{\bar{p}}$

p = power at stimulated frequency

$\bar{p} = \bar{x}$ power at ± 3 db sideband frequencies

are best conducted under conditions where the influence of such factors as the type and level of daily activities, diet and biochemical status can be evaluated and incorporated into the total analysis scheme.

2. Psychomotor function: Results are summarized in Table 8.

a. Eye-hand coordination: The PPEB single axis tracking task was used to assess this function. Mean RMS error was used as the dependent variable. A significant order by drug interaction ($F = 21.36$, 1,22, $p < .001$) consistent with practice effects, was found.

b. Motor coordination: The two-hand coordination task, like the tracking task, resulted in an order by drug interaction ($F = 21.74$, df 1,22, $p < .001$), suggesting that performance improved over time regardless of drug condition.

c. Hand steadiness: Significant drug by day and order by drug by day interactions were found ($F = 3.65$, df 2,44, $p < .05$ and $F = 3.43$, df 2,44, $p < .05$, respectively). As shown in Figure 3, the results cannot be explained solely by practice effects, although practice does have some effect on the performance observed. Steadiness is enhanced by the ingestion of pyridostigmine; subjects who received placebo first showed this effect on both Day 4 and Day 5, while subjects who received pyridostigmine the first week showed enhanced performance only on Day 5.

d. Grip strength: As expected, grip strength was significantly greater for the dominant hand ($F = 45.56$, df 1,22, $p < .001$). A trend toward an interaction between drug, day and hand ($F = 3.30$, df 2,44, $p < .06$) suggested that, on Day 5 of the drug week, grip strength of the dominant hand was reduced. Paired t tests between drug and placebo conditions did not support this hypothesis.

TABLE 8

SUMMARY OF PSYCHOMOTOR PERFORMANCE DATA

Variable	Day	Mean Performance Scores: Pyridostigmine	Placebo	F	MS_{error}	df	p
Tracking (x̄ RMS Error)	4	5.3	5.2				
	5	5.0	4.9				
	8	5.0	5.0				
	Across Days	5.1	5.0	.38	.40	1,22	n.s.
2-Hand Coordination (seconds on target)	4	58.4	58.6				
	5	59.0	58.8				
	8	59.0	59.1				
	Across Days	58.8	58.8	.08	1.95	1,22	n.s.
Hand Steadiness (x̄ Error [sec])	4	6.6	6.4	Drug by Day Interaction			
	5	5.4	6.8	3.65	3.45	2,44	.05
	8	7.2	6.6	Drug Main Effect			
	Across Days	6.4	6.6	.10	4.72	1,22	n.s.
Grip Strength (kg) (Dominant Hand)	4	49.1	47.9				
	5	48.3	48.9				
	8	49.3	49.2	Drug by Day by Hand Interaction			
	Across Days	48.9	48.7	3.30	2.88	2,44	.06
Non-Dominant Hand	4	43.0	43.4	Drug Main Effect			
	5	43.9	43.9	.10	13.14	1,22	n.s.
	8	43.4	42.9				
	Across Days	43.4	43.4				
Simple Reaction Time (msec)	4	229.2	228.8				
	5	216.0	219.6				
	8	215.1	221.2				
	Across Days	220.1	223.2	.53	663.0	1,22	n.s.

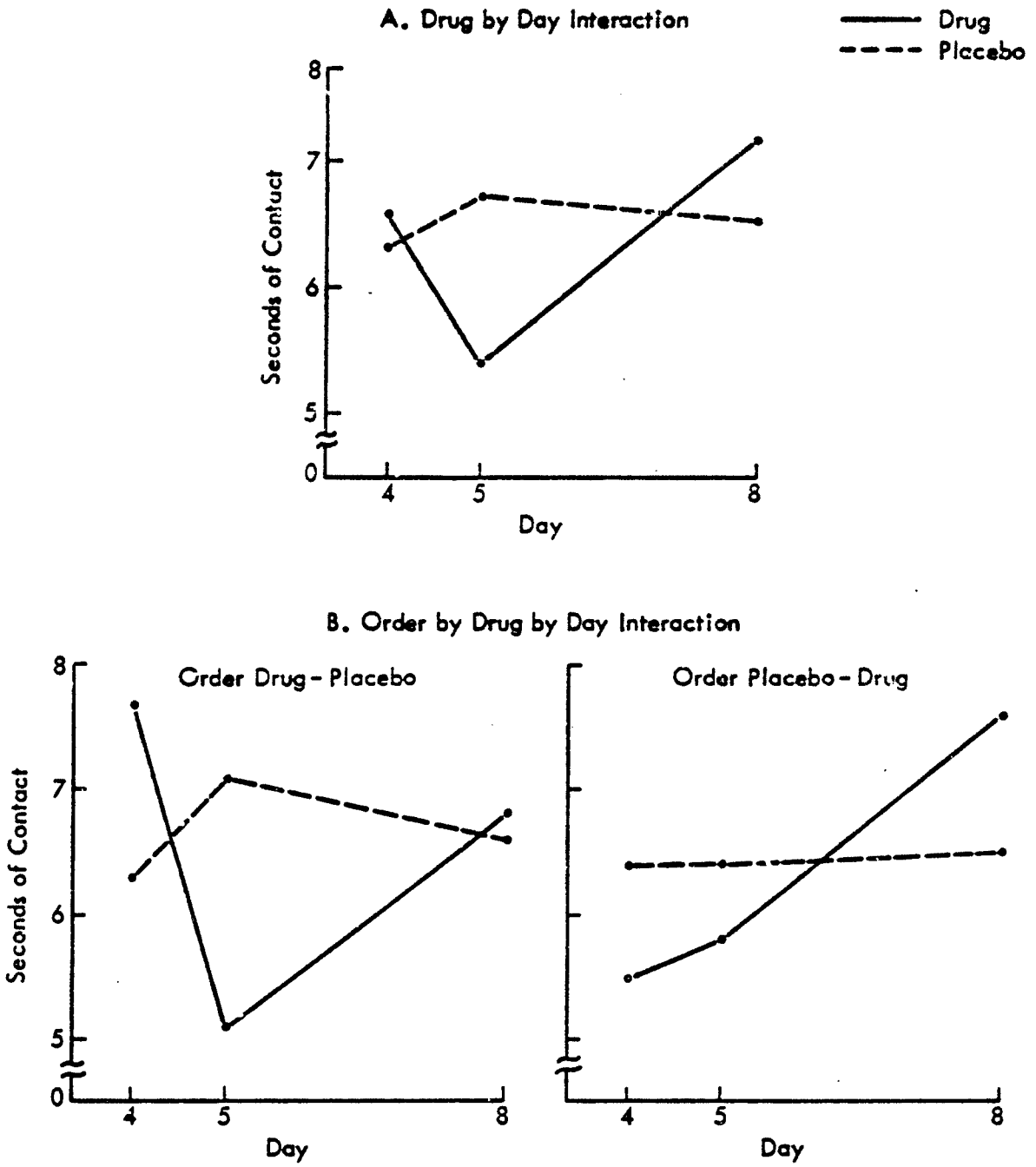

Figure 3 - Significant Interactions Between Drug and Day, and Between Order, Drug and Day for Hand Steadiness.

e. Perceived Exertion Scale: After each grip strength measurement, the subject was asked to rate the amount of exertion expended. There were no significant main effects, although a trend toward a drug by day interaction was observed ($F = 3.14$, df 1,22, $p = .055$). Exertion ratings were lower on Day 5 of the drug regimen than on Day 5 of the placebo regimen. This result suggested that the grip strength data should be reanalyzed, using exertion ratings as the covariate. When exertion ratings were taken into account, the interaction between drug, day and hand was reduced to $F = 3.12$, $p < .07$; changes in perceived exertion do not, therefore, fully account for the differences observed in grip strength.

f. Simple reaction time: No differences in reaction time between pyridostigmine and placebo conditions were found ($F = .53$, df 1,22, $p = > .20$).

3. Central processing: Results for central processing variables are summarized in Table 9.

a. Internal timing: The Interval Production Task was used to evaluate changes in internal timing. No differences attributable to pyridostigmine were found.

b. Memory: No significant differences in memory as measured by the Digit Span Task were found. Analyses of the Sternberg Memory Task resulted in the expected linear relationship between set size and reaction time; pyridostigmine did not alter these functions.

c. Attention: The "3-Meter Monitoring" subtask of the Multiple Task Performance Battery was used to measure attention; attentional interference was assessed by the Stroop Color/Word Task, and perseveration by the Reverse Tapping Task. No significant differences were found for probability monitoring or warning light monitoring. Monitoring of the blinking meter resulted in a significant main effect for days ($F = 5.45$, df 2.44, $p < .01$), and significant interaction between order, drug and days ($F = 3.55$, df 2.44, $p < .05$). Figure 4 presents these results. Performance improves over days, consistent with a practice effect.

TABLE 9

SUMMARY OF CENTRAL PROCESSING DATA

Variable	Day	Mean Performance Score: Pyridostigmine	Mean Performance Score: Placebo	F	MS_{error}	df	p <
Internal Timing (IPT Score)	4	15.5	15.3				
	5	15.8	17.6				
	8	13.7	14.2				
	Across Days	15.0	15.7	1.01	18.78	1,22	n.s.
Digit Span-Forward (Number of Digits)	4	7.5	7.4				
	5	7.4	7.8				
	8	8.0	7.8				
	Across Days	7.6	7.7	.07	.41	1,22	n.s.
Digit Span - Backward (Number of Digits)	4	6.3	6.5				
	5	6.4	6.5				
	8	6.5	6.7				
	Across Days	6.4	6.6	1.16	1.01	1,22	n.s.
Sternberg Memory Task (sec)	4	.329	.360				
	5	.315	.328				
	8	.304	.325				
	Across Days	.316	.338	1.74	.01	1,22	n.s.
Monitoring Reaction Time Probability (sec)	4	13.7	13.0				
	5	12.6	12.4				
	8	12.7	12.7				
	Across Days	13.0	12.7	.18	20.09	1,22	n.s.
Warning (sec)	4	.775	.810				
	5	.859	.814				
	8	.862	.824				
	Across Days	.832	.816	.17	.06	1,22	n.s.
Blinking (sec)	4	2.8	2.5				
	5	2.4	2.3				
	8	2.4	2.3				
	Across Days	2.5	2.4	2.53	.37	1,22	.15

TABLE 9 (Concluded)

Variable	Day	Mean Performance Score: Pyridostigmine	Mean Performance Score: Placebo	F	MS_{error}	df	p <
Stroop Color-Word Test (Reaction Time [msec])	4	788	759				
	5	740	750				
	8	741	726				
	Across Days	756	745	.18	.02	1,22	n.s.
Stroop Color-Word Test (% Correct)	4	95.9	96.2				
	5	95.0	96.5				
	8	96.0	96.0				
	Across Days	96.0	96.2	.37	7.54	1,22	n.s.
Reverse Tapping Task (% Correct)	4	95.8	93.8				
	5	97.2	96.4				
	8	96.8	97.4				
	Across Days	96.6	95.9	1.79	11.15	1,22	.20
2-Digit Addition (% Correct)	4	92.4	94.8				
	5	97.2	96.3				
	8	93.8	95.2				
	Across Days	94.5	95.4	.95	35.77	1,22	n.s.
Grammatical Reasoning (Task Time [sec])	4	123.2	122.6				
	5	120.4	124.8				
	8	116.4	119.0				
	Across Days	120.0	122.1	.30	568.98	1,22	n.s.
Grammatical Reasoning (Number Correct)	4	31.0	31.4				
	5	31.5	31.2				
	8	31.0	31.4				
	Across Days	31.2	31.3	.54	1.05	1,22	n.s.
Forced Choice Reaction (Time sec)	4	.309	.316				
	5	.316	.313				
	8	.318	.306				
	Across Days	.314	.311	.07	.004	1,22	n.s.

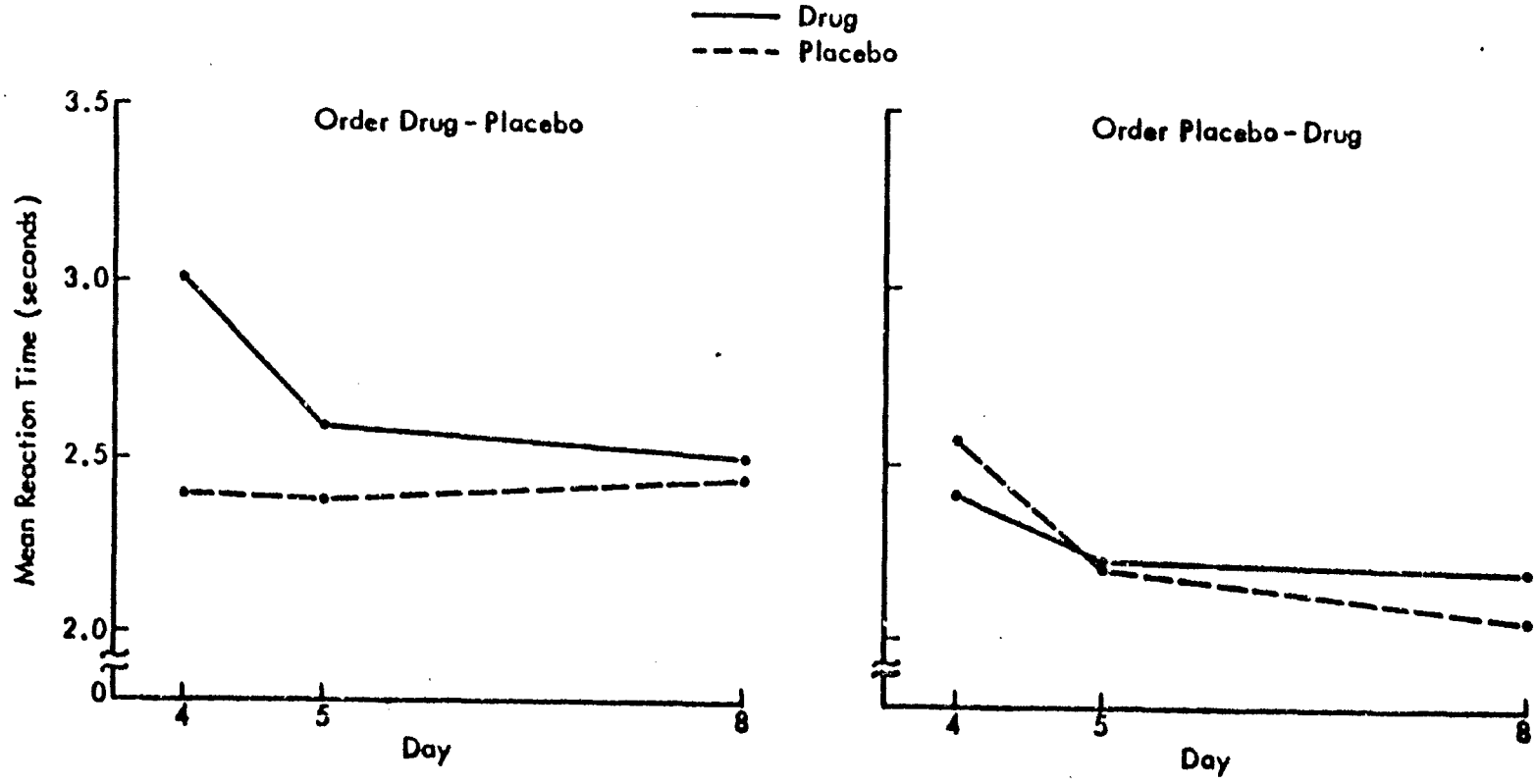

Figure 4 - Interaction Between Order of Drug Administration, Drug and Days for Reaction Time to the Blinking Light Monitoring Task.

Over and above these practice effects, there appears to be a small initial decrement in reaction time to the blinking light monitoring task associated with ingestion of pyridostigmine, particularly for those subjects who received pyridostigmine during the first week.

The Stroop Color-Word Task, which measures attentional interference, provides three measures: percent correct, reaction time, and number of stimuli presented. No significant effects attributable to pyridostigmine were found for any of the three dependent variables.

Performance on the Reverse Tapping Task improved over days ($F = 5.78$, df 2.44, $p < .05$); no significant difference between pyridostigmine and placebo was found.

d. Information processing: The two-digit addition task was used as a measure of symbolic information processing. Accuracy of addition was not different under placebo and pyridostigmine conditions.

e. Decision-making: The Baddeley Grammatical Reasoning task resulted in a significant drug x order interaction ($F = 6.65$, $p < .025$) suggestive of a practice effect. No significant effects were found for forced-choice reaction time.

4. Simultaneous central processing: To assess higher level, simultaneous central processing, three sets of dual tasks were performed. Results are summarized in Table 10.

The Multiple Task Performance Battery allowed the combination of monitoring performance with target identification. Reaction time for all three meter tasks was significantly faster under single task conditions. No significant main effects for pyridostigmine versus placebo were found for the monitoring tasks. However, both probability monitoring and blinking light monitoring resulted in significant Drug by Day by Task interactions which are shown in Figure 5. For both tasks, dual performance on Day 4 was better after ingestion of pyridostigmine.

TABLE 10

SUMMARY OF SIMULTANEOUS CENTRAL PROCESSING RESULTS

Variable	Day	Mean Performance Score: Pyridostigmine Single	Pyridostigmine Dual	Placebo Single	Placebo Dual	F	MS_{error}	df	p <
3-Meter Monitoring (primary) Probability (sec)	4	13.7	15.1	13.0	18.8				
	5	12.6	16.6	12.4	15.4	Drug by Day by Task Interaction			
	8	12.7	15.0	12.7	14.4	3.30	25.91	2,44	.04
	Across Days	13.0	15.6	12.7	16.2	Main Effect for Drug .04	39.98	1,22	n.s.
Warning (sec)	4	.775	1.073	.810	1.128				
	5	.859	.979	.814	1.069				
	8	.862	1.000	.824	.978				
	Across Days	.832	1.019	.816	1.058	Main Effect for Drug .12	.08	1,22	n.s.
Blinking (sec)	4	2.77	2.76	2.49	3.15				
	5	2.40	2.81	2.30	2.56	Drug by Day by Task Interaction			
	8	2.35	2.62	2.25	2.46	4.17	.30	2,44	.03
	Across Days	2.51	2.73	2.35	2.72	Main Effect for Drug 1.31	.43	1,22	n.s.
Target Identification (secondary) (% Correct)	4		78.6		83.0				
	5		70.4		76.8				
	8		78.8		84.2				
	Across Days		75.9		81.3	Main Effect for Drug 1.77	581.3	1,22	.20

TABLE 10 (Concluded)

Variable	Day	Mean Performance Score: Pyridostigmine Single	Pyridostigmine Dual	Placebo Single	Placebo Dual	F	MS_{error}	df	p <
Stroop Color/Word Task (primary) Reaction Time (sec)	4	.79	2.04	.76	2.00				
	5	.74	1.94	.75	1.86				
	8	.74	1.81	.73	1.83				
	Across Days	.76	1.93	.75	1.90	Main Effect for Drug .37	.11	1,22	n.s.
Stroop Color/Word Task (primary) (% Correct)	4	95.9	95.3	96.2	94.6				
	5	96.0	96.1	96.5	95.9	Drug by Task Interaction 5.78	6.62	1,22	.025
	8	96.0	97.2	96.0	94.6				
	Across Days	96.0	96.2	96.2	95.0	Main Effect for Drug 1.91	7.68	1,22	.18
2-Digit Addition (Secondary) (% Correct)	4	92.4	85.0	94.8	88.0				
	5	97.2	90.2	96.3	92.5				
	8	93.8	88.2	95.2	92.1				
	Across Days	94.5	87.8	95.4	90.9	Main Effect for Drug 5.39	53.76	1,22	.03
Single Axis Tracking Task (Primary) (x RMS error)	4	5.26	6.09	5.22	6.14				
	5	4.99	6.04	4.88	5.90				
	8	5.05	5.96	4.99	5.86				
	Across Days	5.10	6.03	5.03	5.97	Main Effect for Drug .47	.64	1,22	n.s.
Sternberg Memory Task (Secondary) (Set Size 6) (sec)	4	.329	.378	.360	.375				
	5	.315	.369	.328	.361	Drug by Task Interaction 3.15	.005	1,22	.09
	8	.304	.375	.325	.361				
	Across Days	.316	.374	.338	.366	Main Effect for Drug .39	.01	1,22	n.s.

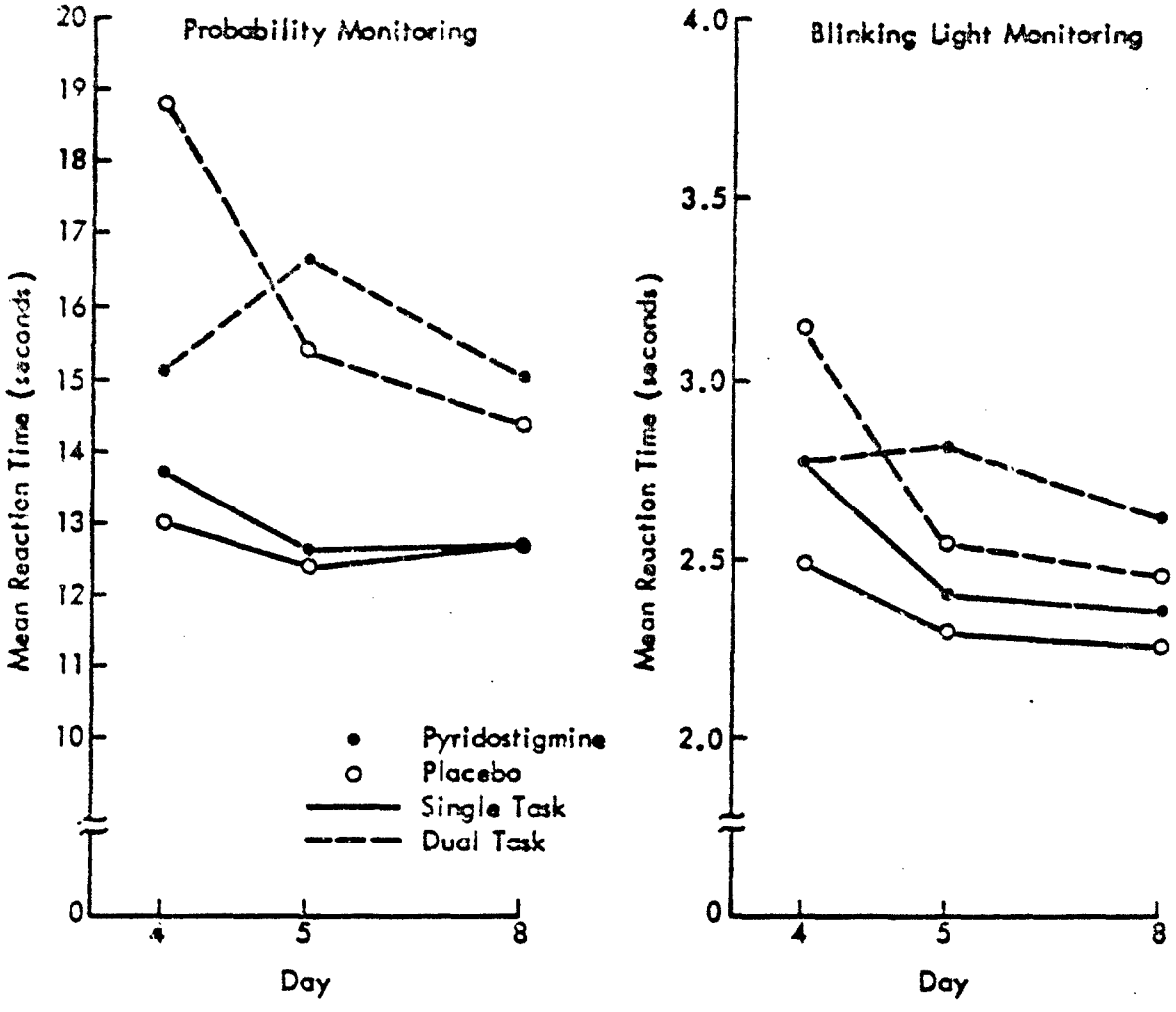

Figure 5 - Drug by Day by Task Interaction Effects for the Visual Probability Monitoring Task and the Blinking Light Monitoring Task.

The Stroop and two-digit addition were combined to evaluate simultaneous attentional and information processing performance. The Stroop served as the primary task. Performance was better under single than under dual task conditions for all measures except for percent correct responses on the Stroop; under pyridostigmine conditions, percent correct responses was not different as a function of single versus dual task performance. Under placebo conditions, the expected decline in performance of the dual task was observed ($F = 5.78$, df 1,22, $p < .025$). The secondary addition task resulted in poorer performance under pyridostigmine than under placebo, ($F = 5.39$, df 1.22, $p < .03$).

To evaluate the combination of eye-hand coordination and memory, the tracking task was performed as the primary task, with the Sternberg as secondary. As expected, tracking performance was better under single than under dual task conditions ($F = 89.49$, df 1,22, $p < .001$), regardless of whether placebo or pyridostigmine was being ingested. The same was true for the Sternberg Memory Task, although the effect of dual task was not so large ($F = 4.70$, df 1,22, $p = .04$). The Sternberg Memory Task was more disrupted by dual performance under the pyridostigmine condition than under the placebo condition (58 versus 28 msec, $F = 3.15$, df 1.22, $p < .09$).

E. Early Effects of Pyridostigmine

In order to evaluate the early effects of pyridostigmine, half of the subjects received an additional test session on Day 2. First, 2 x 2 ANOVAs were performed to determine whether performance on Day 2 was different under pyridostigmine than under placebo conditions. Based on this preliminary analysis, three variables were selected for more detailed evaluation. Table 11 summarizes the results of these analyses. Under dual task conditions, there was a trend for tracking performance on Day 2 to be better under pyridostigmine than placebo ($F = 8.90$, df 1,10, $p < .10$). Probability monitoring was adversely affected by pyridostigmine on Day 2 ($F = 8.90$, df 1,10, $p < .05$); analysis including other test days did not, however, result in a significant main effect for drug. No main effect for drug was seen for the

TABLE 11

SUMMARY OF EARLY* EFFECTS OF PYRIDOSTIGMINE

Variable	Mean Performance Score: Pyridostigmine	Mean Performance Score: Placebo	F	MS_{error}	df	p
Tracking ($\bar{x}$ RMS error)	5.46	5.75	3.59	.27	1,10	.10
Probability Monitoring (reaction time - sec)	17.6	13.9	8.90	17.70	1,10	.05
Warning Light Monitoring (reaction time - sec)			Drug by Task Interaction			
Single Task	.79	.96	4.24	.05	1,10	.10
Dual Task	1.19	1.08				

* Tested on Day 2 of each drug regimen.

warning monitoring task; a trend toward an interaction between drug and single versus dual task was observed ($F = 4.24$, df 1,10, $p < .10$), and suggested that the difference between the two was greater under pyridostigmine than under placebo. Early effects appear to be minimal, although ambiguous vigilance type tasks may be more sensitive to early drug effects than other central processing tasks.

F. Effects of Individual Differences in Cholinesterase Inhibition on Performance

The individual differences observed in cholinesterase inhibition suggested that examining the impact of such differences on performance would be of particular value. Since the experiment was not designed with such analyses in mind, several trade-off decisions were required.

Analysis of covariance was the first strategy considered. The fact that no baseline performance measures were available, and that blood samples were not taken at the same time as performance measures, argued against this strategy. We also did not expect absolute differences in cholinesterase level to predict performance; rather, we anticipated that changes in cholinesterase would be the operative variable, and the data were inadequate to directly address that question using analysis of covariance. A second strategy was to select subjects with high and low (or no) cholinesterase inhibition, and repeat the statistical analyses to determine whether the groups differed in performance. This strategy, by itself, was not considered adequate. A third approach was to perform a regression analysis for each variable to determine how well changes in performance could be predicted by changes in cholinesterase level.

A combination of the last two strategies was implemented. For each individual, two cholinesterase inhibition scores were calculated: percent inhibition related to baseline levels (D-BL/BL), and percent inhibition as compared to levels during the placebo week (P-D/P). Physiological and performance percent change scores were computed on Day 5 data by the

formula Placebo-Drug/Placebo x 100. Because so many variables showed a practice effect, order (drug-placebo or placebo-drug) and a "practice" score (the number of sessions prior to Day 5 of the pyridostigmine week) were used as predictor variables. The practice score ranged from 1 (order drug-placebo, group with no Day 2 testing) to 6 (order placebo-drug, group with Day 2 testing). Mean baseline cholinesterase levels were also included in the regression equation. Stepwise regression was performed using the Statistical Package for the Social Sciences program.

Table 12 summarizes the regression analysis results. Only those variables significantly predicted by one of the two cholinesterase inhibition scores or by baseline cholinesterase are listed. It should be noted that only the depth perception task showed significant effects in the original analysis, or in the analysis of acute response to the drug. The correlation between the two inhibition scores was 61 which, while statistically significant ($p < .01$), represents covariance of only 36%.

As shown in Figure 6, oral temperature change increased as cholinesterase inhibition increased. Data for visual acuity is presented in Figure 7. The greater the cholinesterase inhibition, the greater the decrement in visual acuity. Of the seven subjects who had visual acuity decrements of 10% or greater, six also had cholinesterase inhibiton of greater than 10%. Conversely, all four subjects with increased cholinesterase levels had improved visual acuity. Figure 8 presents the data from the depth perception task. Depth perception performance improved as cholinesterase inhibition increased.

Both cholinesterase inhibition and order predicted the change in total symptoms reported. As cholinesterase inhibitions increased, symptoms also increased. Inclusion of order of drug administration in the model improved the percent variance explained from 22% to 36%. The results are shown graphically in Figure 9. The strong relationship between cholinesterase inhibition and symptom scores is due almost entirely to subjects who received pyridostigmine during the first week. Examination of the residual plot from the regression analysis suggests that some additional variable not included in the model is exerting a significant effect.

TABLE 12

SUMMARY OF SIGNIFICANT REGRESSION ANALYSIS RESULTS

Variable	Predicted by*	Multiple r	Percent Variance Explained	p =
Oral Temperature	$\frac{P-D}{P}$	.49	24	.02
Visual Acuity	$\frac{D-BL}{BL}$	.56	32	.004
Depth Perception	$\frac{P-D}{P}$	.46	21	.02
Total Symptom Score	$\frac{D-BL}{BL}$	.47	22	.02
Two-Digit Addition (single task)	Baseline ChE	.52	27	.009
Grammatical Reasoning	Baseline ChE	.46	21	.02
Diastolic Blood Pressure	Baseline ChE	.47	22	.02

* P = Placebo.
D = Drug.
BL = Baseline.
ChE = Cholinesterase.

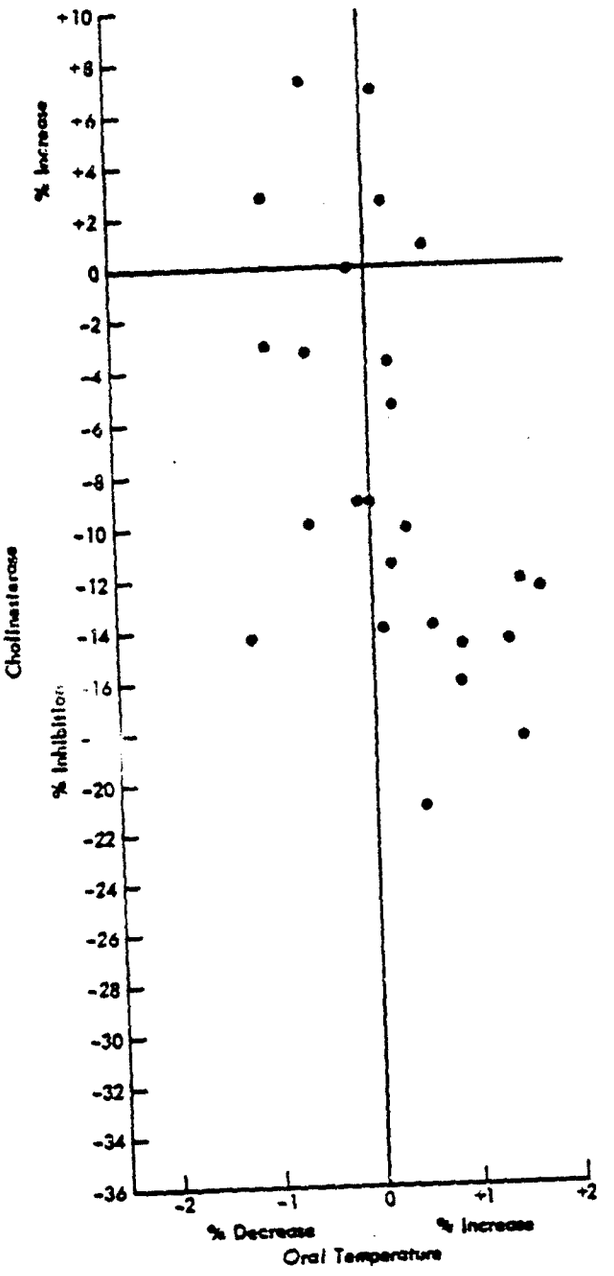

Figure 6 - Relationship Between Cholinesterase Inhibition and Change in Oral Temperature.

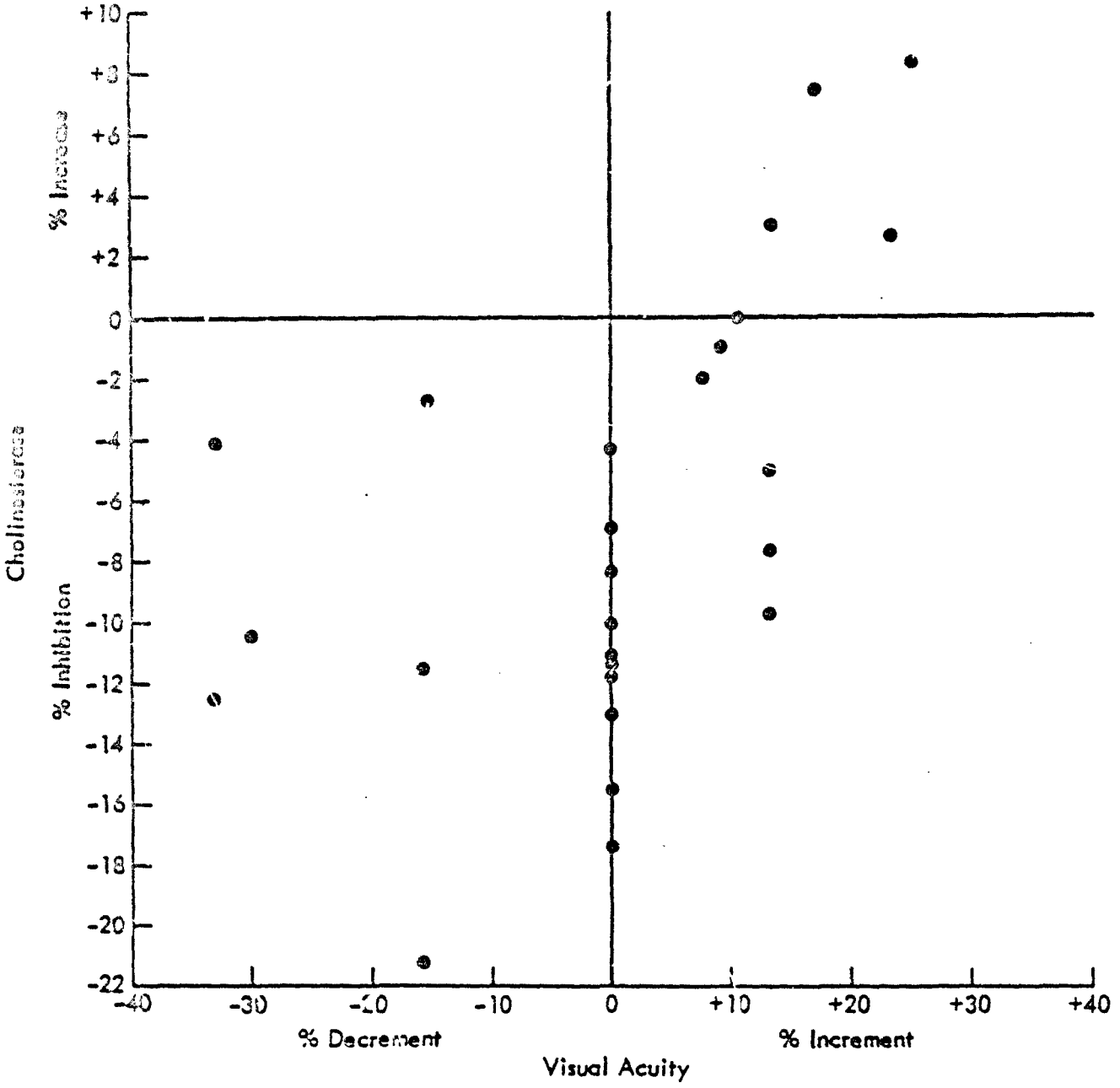

Figure 7 - Relationship Between Cholinesterase Inhibition and Change in Visual Acuity.

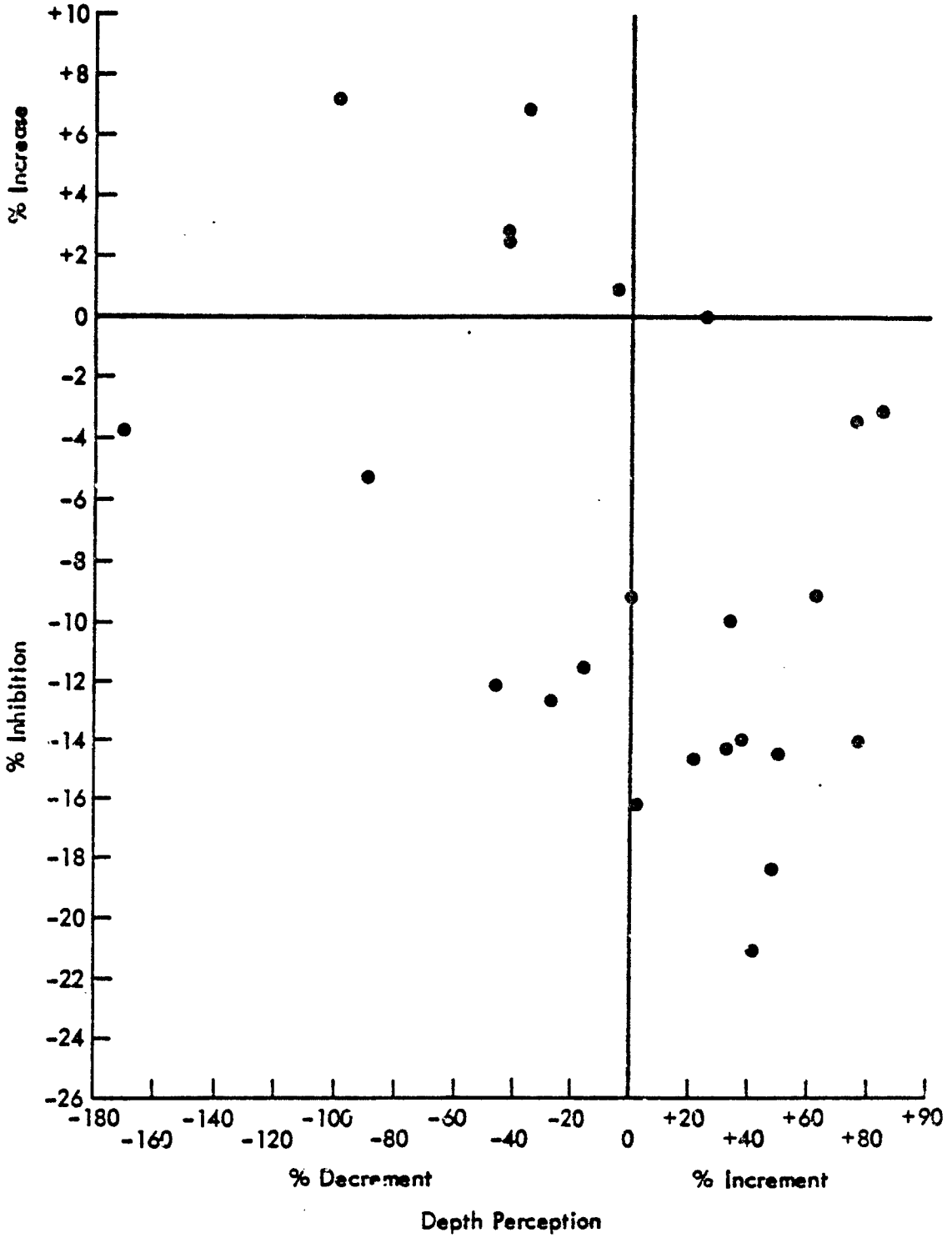

Figure 8 - Relationship Between Cholinesterase Inhibition and Change in Depth Perception.

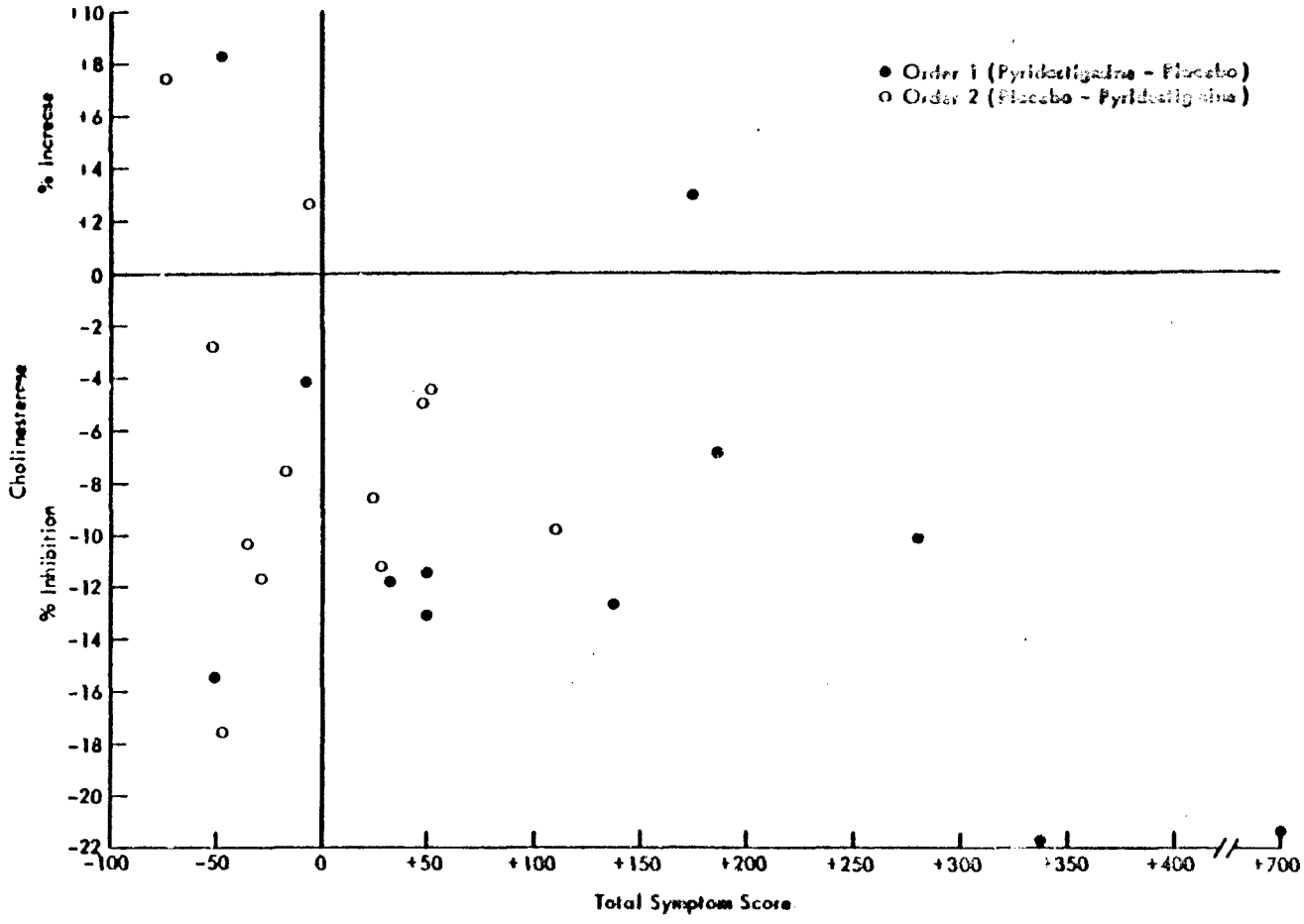

Figure 9 - Relationship Between Cholinesterase Inhibition and Change in Symptom Score.

Baseline cholinesterase levels were not significantly correlated with either of the two measures of ChE inhibition, but did predict changes in diastolic blood pressure, 2-digit addition, and accuracy on the Grammatical Reasoning Task. Examination of the residuals for the addition task suggested that the regression model was inaccurate. Only 25% of the subjects showed performance changes in this variable. The results of the regression analysis for this variable appear, therefore, to be spurious. Figure 10 presents the relationship between baseline cholinesterase and diastolic blood pressure changes in response to pyridostigmine. The greater the cholinesterase level, the greater the percent decrease in diastolic blood pressure. Changes in accuracy on the Grammatical Reasoning Task are shown in Figure 11, which suggests that accuracy decreases as cholinesterase level increases. However, the large number of subjects who show no change in performance accuracy appear to be equally distributed along the range of cholinesterase levels, suggesting that some third variable is exerting considerable influence.

The effects on performance of individual differences in cholinesterase inhibition were also examined by making direct comparison of groups. As was noted earlier, two measures of cholinesterase inhibition were available. Using the Drug-BL/BL score, two groups of subjects were selected: those six subjects with the largest inhibition scores (-12.6% to -21.7%) and those six with smallest inhibition scores (+8.3% to -4.1%). Four subjects in the latter group actually showed increased cholinesterase levels after ingesting pyridostigmine. Independent groups t-tests were performed between those groups using the performance change scores for all variables. The process was repeated using the Placebo-Drug/Placebo score (21.0% to 14.3% versus -7.2% to 0.1%). Results are summarized in Table 13. All probabilities of .20 or less are listed. Since the direction of the change score depends upon the original scoring algorithm, the group showing the largest performance decrement is marked with an asterisk. The results of the comparisons between high and low inhibition groups, despite the simplicity of the analysis and the small number of subjects, strengthen the interpretation of the regression analysis, and suggest functions on which further research might usefully focus.

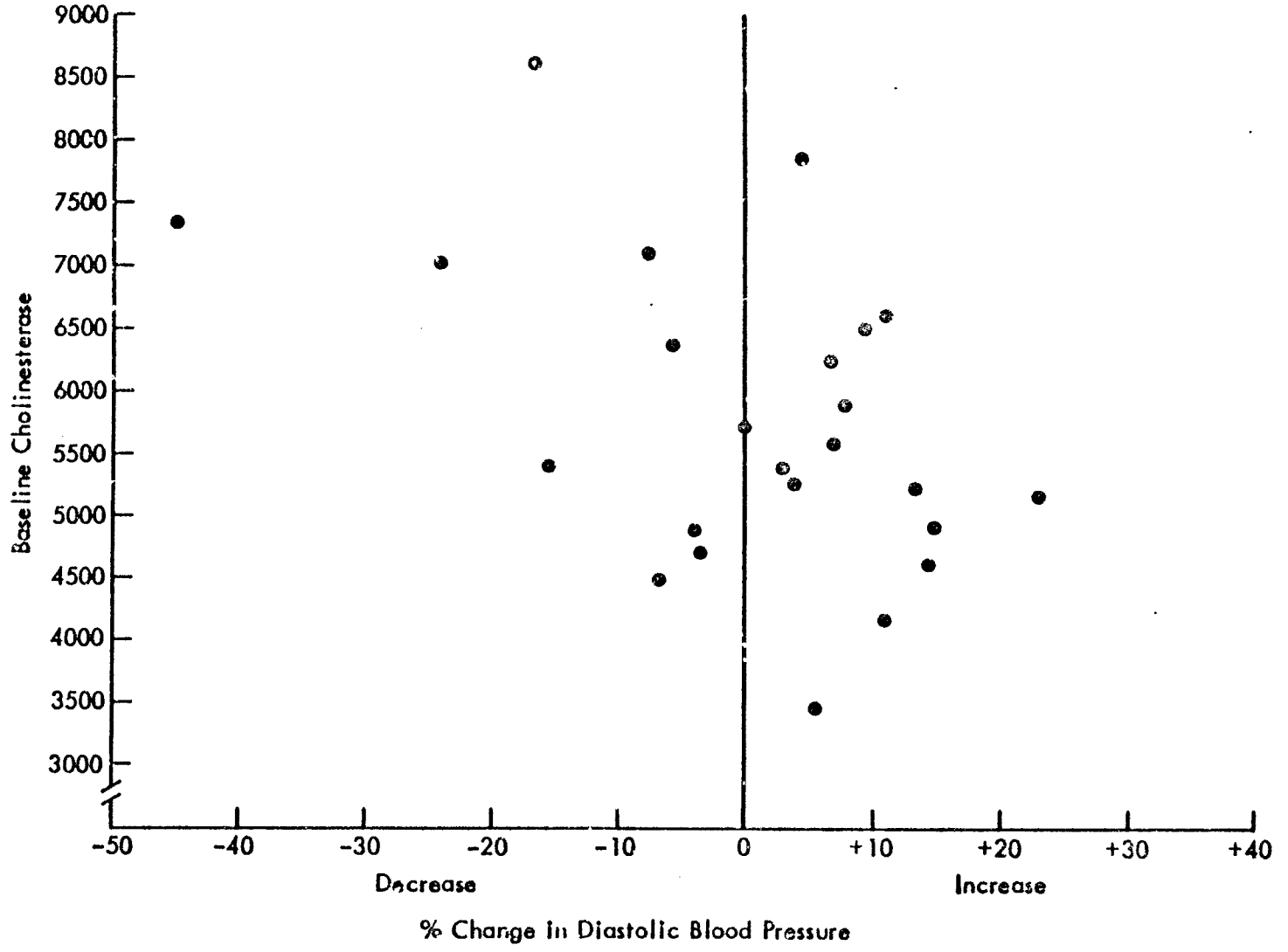

Figure 10 - Relationship Between Baseline Cholinesterase and Diastolic Blood Pressure Changes.

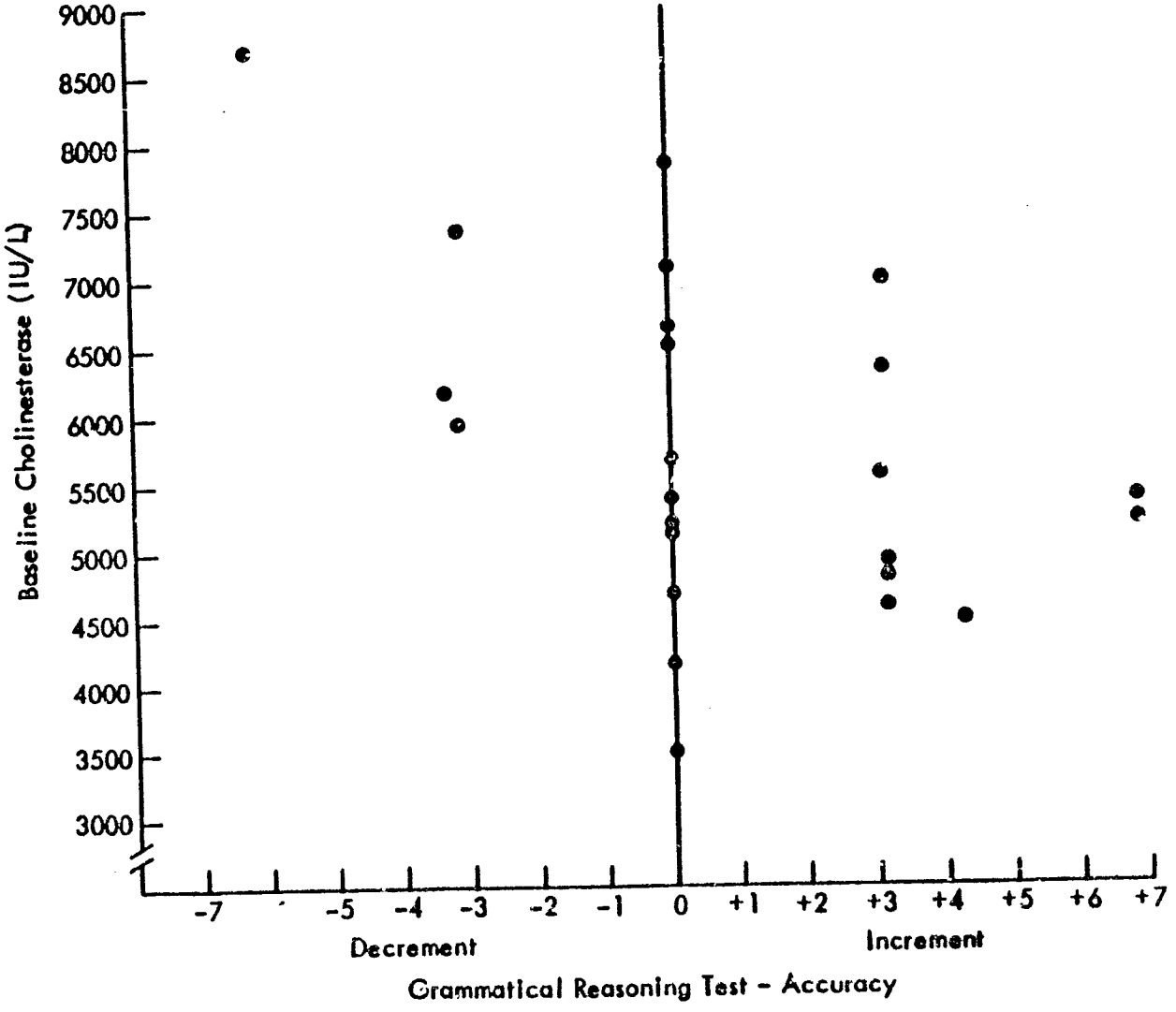

Figure 11 - Relationship Between Baseline Cholinesterase and Change in Accuracy of Performance on the Grammatical Reasoning Task.

TABLE 13

SUMMARY OF COMPARISONS BETWEEN HIGH AND LOW CHOLINESTERASE INHIBITION GROUPS

Variable[a]	Percent Change: High Inhibition $\bar{x}$	High Inhibition S.D.	Low Inhibition $\bar{x}$	Low Inhibition S.D.	t	df	p =
Using Drug-BL/BL to Define Groups							
Oral Temperature	- .90	.64	+ .08	.60	2.75	10	.020
Visual Acuity	- 13.12*	15.58	+ 4.90	23.73	1.55	10	.151
Digit Span Backwards	- 15.40	25.14	+ 6.25*	17.78	1.72	10	.116
Blinking Monitoring	- 23.37*	36.1	+ 6.50	24.10	1.69	10	.123
Tracking Dual	- 16.05*	6.92	- 2.08	18.56	1.73	6.36	.135
Sternberg Memory Task, Reaction Time	- 20.95*	29.79	7.97	17.28	2.06	10	.067
Stroop (% Correct)	2.90*	1.88	0.28	2.37	- 2.12	10	.060
Target Identification	- 33.38	90.32	23.00*	30.07	1.45	6.09	.197
Using P-D/P to Define Groups							
Oral Temperature	- .67	.98	+ .15	.58	1.75	10	.111
Depth Perception	+ 32.57	17.97	- 33.98*	41.27	- 3.62	10	.005
Simple Reaction Time	- .05*	2.87	+ 5.70	6.57	1.96	10	.078
Grip Strength	- 2.03	6.56	+ 5.85*	9.56	1.66	10	.127
Sternberg Memory Task, Dual, Reaction Time	+ 10.47	24.54	- 10.40*	12.42	- 1.86	10	.093

* Group demonstrating greatest performance decrement.

a P = placebo, D = drug, BL = baseline.

G. Task Battery Evaluation

The purpose of this section is to evaluate the preliminary task battery developed for this research program. The evaluation process was conducted on two levels. First, on a general level, each sub-task in the battery was evaluated according to the criteria presented in Table 14. These particular criteria were selected because they provide valuable "generic" informaton for the eventual development of an extensive "menu" of standardized tasks and procedures useful in assessing the effects of a wide variety of pretreatment and prophylactic drugs. Thus, the general evaluation process examined the preliminary task battery without reference to the sensitivity with which any particular sub-task reflected the specific effects of pyridostigmine.

The second part of the evaluation process examined the unique capacity of the preliminary battery to reflect the effects of pyridostigmine as tested in this research program. Thus, the specific evaluation process took into account the particular experimental design followed, the unique subject sample tested, and the actual performance data obtained. A number of sub-tasks in the battery showed no difference in performance under pyridostigmine compared to placebo conditions. Under these circumstances, a major question concerned the degree to which we could place confidence in the findings of no difference. In other words, was there really no difference, or was the sub-task or the sample size simply not adequate to detect a difference if it did exist? An appropriate statistical technique to answer this question is Power Analysis (Cohen, 1977). This technique, and the findings obtained through its application, are described in detail in Section G.2. below.

1. General evaluation: Ideally, any multi-task battery should be composed of sub-tasks that meet the following criteria: (1) each sub-task should have a known testing history and/or specific relevance for the particular human function of interest; (2) task training time should be relatively brief; (3) once a subject is trained, his performance should be stable; and (4) when the task is used experimentally, it should have the capacity to reflect both increments and decrements in performance.

TABLE 14

SUMMARY OF PERFORMANCE BATTERY EVALUATION

Task	Evaluation Criteria*				Comments
	Task Relevance	Training Time	Performance Stability	Performance Bimodality	
VISUAL FUNCTION					
Contrast Sensitivity	1	3	4	2	Even after considerable training, data were highly variable and appeared to be very sensitive to brief lapses of attention. Alternative testing procedures should be used (e.g., method of manual adjustment).
Steady State VER	2	2	4	?	EEG following was variable; unable to derive and evaluate neural transit time measures.
Visual Acuity	1	1	3	3	Better methods should be used; eye charts not adequately sensitive.
Depth Perception	1	2	3	2	More sensitive methods should reduce variability.
PSYCHOMOTOR FUNCTION					
Two-hand Coordination	1	4	3	4	Nature or the task implies either a practice effect or a ceiling effect.
PPEB Tracking Task	1	4	3	2	Nature of the task implies either a practice effect or a ceiling effect; adaptive tracking task might prove more sensitive.
Stabilimeter	1	1	2	2	Good task, but requires careful supervision of subjects during testing.
Simple Reaction Time	1	2	1	1	Good task.
Grip Strength	1	1	2	2	Good task; requires close supervision.
Exertion Scale	2	1	2	2	Good scale; further validation for exertion of small muscle masses would be valuable.
CENTRAL PROCESSING					
Interval Production Task	2	3	3	2	Increased training time might decrease variability observed.

TABLE 14 (concluded)

Task	Evaluation Criteria*				Comments
	Task Relevance	Training Time	Performance Stability	Performance Bimodality	
Digit Span	1	1	2	2	Most disliked task in battery.
Sternberg Memory Task	1	2	2	2	Task should be reprogrammed to provide more detailed data, and to simplify training.
3-Meter Monitoring	1	3	2	2	Task should be reprogrammed to simplify training and improve data retrieval. Subjects judged this the most boring task in the battery.
Stroop Color-Word Task	1	2	3	2	Training time should be increased; task as programmed lacks standard control conditions; should be experimenter-paced.
Reverse Tapping Task	2	3	2	3	Task was too easy for this subject sample.
Two-Digit Addition	1	2	?	1	Task was too easy for this subject sample.
Grammatical Reasoning Task	1	2	3	2	Not suitable in present form for extensive repeated-measures testing; should be computerized to allow more detailed analyses of performance.
Forced-Choice Reaction Time	1	1	2	2	Task should be reprogrammed or redesigned to eliminate scoring errors.
SIMULTANEOUS TASKS					
3-Meter Monitoring with Target Identification	1	3	2	2	Not optimal for brief presentation; does not produce sufficient data points.
Stroop Color-Word Task with Addition	1	2	3	1	Secondary task was too easy for this subject sample.
Tracking with Sternberg Memory Task	1	2	2	2	See comments for single tasks.

* 1 = Excellent; 2 = Good; 3 = Fair; 4 = Poor; ? = see comments.

As can be seen in Table 14, the sub-tasks developed for the preliminary battery met the above criteria in varying degrees. A majority of the tasks selected were standardized tasks widely used in previous research contexts to evaluate drug action and/or functions important in pilot operations. Thus, in terms of testing history and functional relevance, the task battery rates quite high.

Performance stability and performance bimodality are intimately related to the level and type of performance training criteria set, and to the amount and adequacy of task training the subjects received. Eleven of the twenty-two tasks presented in Table 14 were rated "good" or "excellent" in terms of performance stability, and eighteen of twenty-two received similar ratings in regard to their ability to show bimodal performance effects. The areas in which the preliminary battery could definitely be improved lie in the time required to train subjects, and in specific performance criteria set for particular tasks.

Subjects spent most of their training time learning to perform the two tracking tasks to criteria. However, despite the large amount of training received, subjects continued to improve in their tracking ability on both tasks over the multiple drug and placebo testing sessions. In the case of the two-hand coordination task, continued skill acquisition occurred in the extreme upper range of task performance (i.e., a "ceiling" effect). In other words, the task was too easy for this subject sample. The Reverse Tapping Task and the Two-digit Addition task showed similar effects.

Of all the tasks in the preliminary battery, the two tracking tasks showed the largest practice effects (ANOVA, order x drug interaction, $p < .001$). A partial explanation for the effects observed lies in the type of training criteria used. Subjects trained to a performance criteria rather than to a certain number of task trials. Thus, subjects with high initial tracking ability performed relatively fewer training trials compared to subjects with low initial ability. Subjects with high initial ability continued to improve during the testing sessions. Changing to an individually-based performance criteria, or using an adaptive type tracking task (e.g., a JEX type task as originally proposed) could markedly improve evaluation.

Practice effects are usually thought of as undesirable, however, they can sometimes be useful in other contexts. For example, in this study subjects were ingesting either pyridostigmine or a placebo during the time they were continuing to learn to perform the tracking tasks. By evaluating the differences observed in the skill acquisition curves obtained under drug versus placebo conditions, it is possible to gain additional information about the effects of a particular drug on the learning or acquisition of a skill.

We conducted a preliminary evaluation of the acquisition curves for the PPEB Tracking Task, taking into account the order in which subjects received the drug or the placebo and whether or not they had additional test sessions to assess acute drug effects. Pyridostigmine had no apparent effect on skill acquisition of PPEB tracking. It should be noted, however, that this was a preliminary analysis conducted on data collected in a study not specifically designed to address this type of question. Other sub-tasks demonstrating significant practice effects were:

Grammatical Reasoning Task	($p < .025$)
Visual Contrast at 0.5 c/d	($p < .05$)
Stroop (mean reaction time)	($p < .05$)
Stroop (No. stimuli presented)	($p < .03$)
Blinking Light Monitoring)	($p < .01$)

Several of the above tasks had unique problems. The Grammatical Reasoning Task consisted of multiple, conuter-balanced versions of a 32-item paper and pencil test. From discussions with the subjects, it became clear that it was relatively easy for them to memorize the answers to particular items, and therefore, to demonstrate an apparent performance improvement over testing sessions. This task could be made more suitable for multiple testing by enlarging the item pool using various symbol sets. In addition, if a computer program was developed to present and time the response to each item separately, performance data could be related to the rationale underlying the task.

The practice effects observed for the two Stroop measures listed above are essentially reciprocals of one another (i.e., if one performs the task more quickly, one automatically performs more task trials). In addition, the computer program used to present the Stroop task did not allow presentation of the traditional control conditions. Inclusion of such control conditions would allow collection of the more definitive difference measures to evaluate drug impact on attentional interference. Specific problems and potential solutions associated with the measurement of Visual Contrast Sensitivity and the Steady State VER are presented in Table 14. Additional, better alternative methods for the measurement of visual function parameters are available. Although these are more expensive they should be included in future battery applications.

In summary, the general evaluation presented in Table 14 and discussed above indicates that a number of the sub-tasks developed for the preliminary battery could prove useful in future evaluations of pretreatment and prophylactic drugs. The problems noted in relation to specific sub-tasks have potentially effective solutions, and should be implemented in future task battery applications. One major outcome of this evaluation process is to demonstrate the need for standardization of task training parameters, task testing protocols, and specific performance measurement selection.

2. Specific evaluation: In evaluating the adequacy of the task battery and the experimental design, it is important to consider the statistical power of the tasks used. Power is the ability of a statistical test to detect an effect if, in fact, an effect exists. Power depends on the size of the effect, the size of the sample, and the alpha level selected for rejection of the null hypothesis. By performing power analysis on data from a completed experiment, one can determine whether the sample size was adequate to detect a true difference. For each of the performance variables, effect size and power of the main effect for pyridostigmine versus placebo were calculated. Table 15 summarizes the results. "Effect size" is the relationship between mean differences and variability; in biobehavioral research, an effect size of .10 or less is considered small, and effect size of .50 or more is considered large. The figure in the power column can be

TABLE 15

POWER ANALYSES FOR MAIN EFFECT OF PYRIDOSTIGMINE VS. PLACEBO

Variable	Effect Size	Power
Serum cholinesterase	.29	> .99
Systolic blood pressure	.06	.28
Diastolic blood pressure	.04	.12
Oral temperature	.08	.45
Pulse rate	.02	< .08
Visual acuity	.02	< .08
Depth perception	.23	.96
Contrast sensitivity - 0.5	.03	< .08
1.0	.08	.29
3.0	.17	.80
6.0	.02	< .08
11.4	.01	< .08
22.8	.04	< .08
Tracking	.08	.29
Two-hand coordination	0	< .08
Hand steadiness	.05	.13
Grip strength	.03	< .08
Simple reaction time	.06	.18
Internal Production Task	.08	.29
Digit span - forward	.08	.29
Digit span - backward	.10	.39
Sternberg Memory Task	.11	.46
Probability monitoring	.03	< .08
Warning light monitoring	.03	< .08
Blinking light monitoring	.08	.29
Stroop color-word, RT	.36	> .99
%	.04	.08
2-Digit addition	.08	.29
Reverse tapping	.1[illegible]	.39
Grammatical reason - time	.04	.08
No.	.05	.13
Forced choice RT	.02	< .08
Probability Monitoring	.48	> .99
Warning light monitoring	.04	.12
Blinking light monitoring	.13	.80
Target identification	.11	.68
Stroop - reaction time	.06	.[illegible]8
% correct	.18	.96
Addition (secondary task)	.27	> .99
Tracking (primary task)	.08	.45
Sternberg (secondary task)	.07	.37

interpreted as the probability of detecting an effect if an effect exists (e.g., power of .80 means that the probability of detecting an effect given the sample size and experimental design used is 80%).

Examination of Table 15 reveals that sample size was quite adequate for several of the tasks which did not change as a function of ingesting pyridostigmine. We can say with confidence that those functions are unaffected by pyridostigmine in the doses used. For many other variables, power is below .80; we cannot, therefore, be sure that an effect did not occur. There are two ways to resolve this problem. One can repeat the experiment using a larger sample size. When the effect is very small, such an approach can be extremely expensive. For example, it would be necessary to have a sample of more than 130 subjects to detect an effect on tracking if an effect exists. The other approach is to increase the effect size by reducing variability. This can be accomplished by selecting other methods for measurement of the function of interest, or by improving the method already used (using a different algorithm to measure performance, increase training times, etc.). It is interesting to note that most of the performance tasks with low power could be criticized on other grounds as well. Further work in the area should take these considerations into account.

IV. DISCUSSION AND CONCLUSIONS

A. Health Effects and Drug-related Symptoms

The pyridostigmine regimen evaluated in the present study was selected, in part, on the basis of previous NATO reports indicating that its use would present only minimal risk to the health of the participants. This judgment was supported. Drug intake was well tolerated by the healthy male participants. No evidence of adverse health effects was associated with participating, or found in daily vital signs. Measures of subjective state, and of daily life and work activities outside the experiment, also failed to distinguish between drug and placebo conditions.

Only one subject reported what he believed were drug-related symptoms (excessive physical fatigue and malaise). This occurred after the subject received four 30-mg pills of pyridostigmine at 8-hr intervals. After monitoring this subject for the following 3 days, and considering all available data, it was the judgment of the staff that the reactions of this subject could not be directly attributed to the effects of the drug (see Appendix A).

Experimenters were not able to distinguish at better than chance levels whether the subjects were receiving pyridostigmine or the placebo. During the first week of drug administration, subjects self-ratings were also no better than chance. They too, could not tell the difference between the drug and the placebo. It was not until the second week of drug administration that subjects began to be able to make this distinction. In the second week, no subject who was taking the placebo judged it to be pyridostigmine.

Interviews were conducted after completion of the study to learn more about the underlying factors involved. Unfortunately, these interviews shed little light; subjects were generally surprised when told their level of accuracy, and no common distinguishing characteristics emerged from the interviews.

The two drug-related symptoms of upset stomach and flatulence reported in the NATO studies, were not replicated in this study. No cluster of drug-related symptoms was apparent. However, evaluation of the daily Symptom Check List developed by MRI for this project, revealed that subjects reported more symptoms when taking pyridostigmine than when taking the placebo. This difference approached statistical significance. It is of interest to note also that a number of subjects reported that they believed pyridostigmine intake enhanced sexual function.

Due to the small number of subjects and the large number of variables in the Symptom Check List, more detailed analyses were inappropriate. The instrument, however, does show promise. It should be more fully developed

for possible use in future research activities or field trials of pyridostigmine and other pretreatment and prophylactic medications.

B. Biological Implications

The drug regimen produced the expected mean level of inhibition in plasma cholinesterase, with values returning to baseline when assessed 64 hr after drug intake ceased. However, subjects showed large individual differences in plasma cholinesterase inhibition. Individual differences in the present study ranged from -21% to +8%.

The observation of individual differences in cholinesterase inhibition raises two sets of relevant questions. The first set concerns the biological impact of pyridostigmine in nonclinical populations, and identification of the factors that influence absorption and excretion of the drug. The second set of questions involves the performance consequences of the individual differences observed in cholinesterase inhibition. This section discusses the initial questions; performance consequences are discussed in Section IV.C.2.

It should be noted that our observation of individual differences in inhibition is not unique. Absorption of pyridostigmine in myasthenia gravis patients has also been reported to be erratic (White et al., 1981). One contributing factor could be individual variation in diet and meal times. For example, Aquilonius et al. (1980), have reported that when a single dose of pyridostigmine is administered to a fasting subject, peak plasma concentration occurs approximately 1.7 hr after intake. In contrast, peak plasma concentration does not occur until approximately 3.2 hr after drug intake in the nonfasting subject. In the present study, neither diet nor meal times were under experimental control, and thus, could have contributed to the variations observed in inhibition.

Additional factors influencing cholinesterase inhibition include individual differences in metabolism, and variation resulting from the particular route and type of drug administration used. Finally, in the present study, it was agreed that assays would be conducted in plasma. The assay procedure used in this study was the Dietz modification of the Ellman procedure. Our evaluation indicated that the particular assay procedure selected for this program demonstrated a high degree of reproducibility. Measures of red blood cell cholinesterase, however, appear to be more stable, sensitive and less variable. In addition, many segments of DOD currently use RBC methods. Consequently, for consistency and greater generalizability from study to study it is recommended that future studies incorporate a preferred method for red blood cell analysis (e.g., Ellman thiocholine).

Given the intended use of this drug, and the large degree of individual differences observed, it is clear that more directed pharmacological studies are called for. For maximum applicability, such studies should be conducted in man, and should include: standardized, baseline biochemical assessments; diet, sleep, activity and physiological monitoring; and detailed pharmacokinetic procedures. Such studies should also take into account the recent findings of Maxwell et al. (1984). These researchers have reported that antidote effectiveness may be a function of the interaction between the degree of cholinesterase inhibition induced, and inter-species variation in endogenous nonspecific tissue binding sites.

C. Performance Consequences of Drug Intake

1. Group effects: Both the early and later effects of the pyridostigmine regimen on performance were examined. Early effects were evaluated using the test results obtained on the second day of drug intake. Subjects performed significantly more poorly on the visual probability monitoring task under pyridostigmine; no other effects were significant.

The later effects of intake were evaluated using the data obtained on days 4 and 5 of drug intake. Both improvements and decrements in performance were observed. Performance under pyridostigmine improved significantly on tests of depth perception, visual contrast sensitivity at 3 c/d, and on tests of hand steadiness.

Few performance decrements were observed; however, those decrements that did occur were in particularly significant areas. Decrements occurred primarily under conditions where the workload on subjects was increased, and they were required to perform two tasks simultaneously.

For example, in one dual task, subjects were required to perform a primary attention task and a secondary mental addition task. Under these conditions, accuracy at the addition task declined significantly more under the drug compared to the placebo condition. Similarly, in another dual task subjects were required to perform a primary visual motor tracking task and a secondary memory search task. The memory search task was more disrupted under the drug compared to the placebo condition.

These findings clearly point to the value of using the dual task strategy in future studies of this type. Under single task conditions, no decrements in central processing functions were found. Under dual task conditions, performance on the primary task was maintained under the drug condition, but only at the cost of declining performance on the secondary task. These findings suggest that pyridostigmine may have a negative impact on the reserve capacity used by an individual when he is required to perform tasks that require rapid time-sharing of attention and cognitive effort.

2. Individual difference effects: The performance consequences of individual differences in cholinesterase inhibition were evaluated using three different procedures. First, regression analyses were performed to assess the relationship between changes in performance and the changes in inhibition. These analyses indicated that as cholinesterase inhibition increases, oral temperature increases, visual acuity declines, and depth perception improves. Second, the predictive value of the subject's initial baseline

level of cholinesterase was evaluated. This analysis indicated that the higher the initial level of cholinesterase on entry into the study, the greater the percent decrease in diastolic blood pressure observed under pyridostigmine.

The final analysis focused on only those subjects who showed either the greatest or the least amount of cholinesterase inhibition. These subjects were divided into a "high inhibition" group and a "low inhibition" group, and group differences in performance were evaluated. This last analysis produced results similar to the above findings; the high inhibition group showed decreases in visual acuity, increases in oral temperature, and improvements in depth perception.

D. Consideration of Performance Findings

It should be noted that there is an apparent paradox in regard to the performance results described above. On the one hand, subjects showed significant decrements on various tasks in the test battery. On the other hand, they apparently had no trouble going about the daily business of living. For example, they could drive cars, go dancing, perform their normal job, do homework, etc., etc. So, the practical question is, just how important or meaningful are the laboratory results reported?

We think the laboratory results are both meaningful and important for the following reasons. First, a distinction needs to be drawn between incapacitation and functional performance decrement. The proposed use of pyridostigmine is as a pretreatment medication, therefore, the drug regimen was deliberately selected so that it would not result in incapacitation. In contrast, the task battery was designed to evaluate the impact of pyridostigmine on specific functions of military importance.

In other words, a decrement in the ability to perform simultaneous tasks can have serious consequences if you happen to be flying an F16 aircraft, and have to make split-second, multiple decisions; however, it may have very little effect if you are driving a car and talking to a friend at the same

time. Similarly, if you are in a situation where you have to wear chemical defense gear, a drug-induced increase in temperature can be significant. If, however, you are performing your duties in an air conditioned office, such an increase is probably of little importance. Finally, a decrease in visual acuity can have a significant impact if you are a photo interpreter; it does not mean that you cannot read the newspaper.

The point of the above examples is that, by definition, pretreatment drugs are designed for two purposes; the first is to provide protection, and the second is to allow the person to continue functioning. Thus, any performance decrements observed are expected to be subtle. A subtle performance decrement can only gain importance and meaningfulness in relation to the specific requirements of the job the individual is asked to perform. If a particular job has requirements that load heavily on specific human functions, and these abilities are reduced by the drug, then performance of that job, and the other jobs that interact with it, may also be reduced.

The practical implication of the above analysis is that a laboratory-based task battery, by itself, is only the initial step in conducting a comprehensive evaluation of the effects of proposed pretreatment and prophylactic drugs. An equally comprehensive task analysis of relevant Military Occupation Specialties needs to be included. It is the relationship between the functional decrements observed in the task battery, and the job requirement profiles of specific military occupations, that allows the true impact of a drug to be evaluated.

A second point to consider is that the performance decrements described above were obtained under fairly ideal laboratory conditions. In other words, the subjects were not stressed, the assessment atmosphere was relaxed and friendly, and the evaluation was conducted under the usual indoor conditions of temperature, pressure and humidity. However, it is a common research finding that as personal, situational, or environmental stress on an individual is increased, performance decrements tend to become both more severe and more apparent. Thus, it is to be expected that pyridostigmine intake under stress conditions might result in a more negative performance

profile than that presented here. For example, Francesconi et al. (1984) recently tested the ability of rats to work in the heat after pyridostigmine intake. Compared to control conditions, pyridostigmine intake results in significantly reduced endurance capacity and compromised thermoregularity efficiency.

Care should be taken, however, when attempting to extrapolate from the findings reported here to the military environment. First, a large number of statistical tests were performed; thus, some of the findings may simply be due to chance. On the other hand, performance decrements were also seen on a number of tasks in addition to those mentioned above. Many of these did not reach appropriate levels of statistical significance, and therefore, are only considered to be "trends."

It is the convention not to discuss such trends. However, strict reliance on statistical significance levels can sometimes provide only a limited picture. An example might make this point more clearly. Say one-third of the subjects in a research study have a 30% reduction in the ability to perform simultaneous tasks, and the rest are unaffected. The results in the laboratory are not likely to reach traditional statistical significance levels. If, however, the same thing happened in a real-world military operation, the consequences could be considerable.

The final point to consider in relation to performance is the adequacy of the task battery developed in this program. A number of task batteries have been developed in the past, and many are in current use in various laboratories around the world. It is important to note, however, that the need for evaluating performance related to pretreatment and prophylactic drugs is relatively recent. Consequently, the existing test batteries are not generally designed for this specific purpose. The battery developed in this program was designed to evaluate the effects of pyridostigmine, and is considered to be only preliminary in nature.

Because of the significant need to develope adequate and effective task batteries in this area, we attempted to provide a comprehensive evaluation of the battery used in the present program. Tables 14 and 15 of this report summarize the results of this evaluation, and the findings should prove useful in future battery development. On the positive side, the present battery rated well on the criteria of sub-task testing history, functional relevance, and the ability to show both increments and decrements in performance. A number of the sub-tasks also demonstrated adequate power to detect a performance change if one was present. However, the evaluation also indicated that improvement was required in the area of training time required, and in the degree of performance stability observed once training was completed.

In conclusion, the findings of the present study indicate that the pyridostigmine regimen evaluated was well tolerated, but resulted in large individual differences in cholinesterase inhibition. While few performance decrements were observed, these occurred in functions of military significance. Additional research is called for in a number of the areas indicated above.

V. REFERENCES

Aquilonius, S. M., S. A. Eckernäs, P. Hartvig, B. Lindström, and P. O. Osterman, "Pharmacokinetics and Oral Bioavailability of Pyridostigmine in Man," European J. Clin. Parmacol., 18, 423-428 (1980).

Blanchard, J., J. Chromatogr., 226, 455-460 (1981).

Chan, K., N. E. Williams, J. D. Baty, and T. N. Calvey, J. Chromatogr., 120, 349-358 (1976).

Cohen, J., Statistical Power Analysis for the Behavioral Sciences, Academic Press, New York, New York (1977).

Ellin, R. L., P. Zvirblis, and M. Wilson, J. Chromatogr., 228, 235-244 (1982).

Francesconi, R., R. Hubbard, and M. Mager, "Effects of Pyridostigmine on Ability of Rats to Work in the Heat," J. Appl. Physiol., 56(4), 891-895 (1984).

Gall, D., "The Use of Therapeutic Mixtures in the Treatment of Cholinesterase Inhibition," Fundamental and Applied Toxicology, 1, 214-216 (1981).

Maxwell, D. M., F. M. Reid, and D. E. Jones, "Correlation of Inter-species Variation in Treatment Efficacy with Endogenous Nonspecific Agent Binding," Preceedings of the 4th Annual Chemical Defense Bioscience Review, Abstract No. 4 (1984).

White, M. C., P. de Silva, and C. W. H. Harvard, "Plasma Pyridostigmine Levels in Myasthenia Gravis," Neurology, 31, 145-150 (1981).

Yakatan, G. J., and J. Tien, J. Chromatogr., 164, 399-403 (1979).

APPENDIX A

STAFF ACTIONS TAKEN IN REGARD TO THE SINGLE SUBJECT WHO REPORTED DRUG SIDE-EFFECTS

INTEROFFICE COMMUNICATION

MIDWEST RESEARCH INSTITUTE

July 15, 1983

To: Members, MRI Human Subjects Committee

From: L. Breed

Subject: MRI Project 2030-09-E, "Effects of Pyridostigmine on Psychomotor and Visual Performance

I feel that the Committee should be informed of the incident described in the attached letter written by Dr. Graham. It seems to me that the problem with the subject was handled in an exemplary fashion by the investigators and subject suffered no injury or no more than temporary ill effects in the experimental procedure. Please call me if you have any questions or feel the incident requires additional Committee scrutiny.

LWB:jh

Distribution:

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July 13, 1983

Capt. Ronald E. Yates
Aerospace Medical Research Laboratory (AFSC)
AFAMRL/HET
Wright-Patterson AFB, OH 45433

Dear Captain Yates:

During the initial drug administration week for the first group participating in our study (Effects of Pyridostigmine on Psychomotor and Visual Performance) one subject reported some difficulties. The purpose of this letter is to inform you of this volunteer's experience and of the actions taken by the project staff.

A joint decision was made by the project staff and the project physician to discontinue the drug regimen for one volunteer (Subject No. 6). This subject received a total of four drug doses, three at 8-hr intervals on day 1 of the drug regimen and one additional pill on the morning of the second drug day. He began reporting excessive physical fatigue and lack of normal endurance during administration of the third drug dose. These symptoms seemed worse to him at the start of drug day 2. His pulse rate and systolic blood pressure were also less than normal for him. He reported that the fatigue he felt was purely physical in nature; he noticed no change in his mental state or level of cognitive function.

We checked with Dr. Diederich, the project physician, who examined the double-blind code list and advised that we discontinue the subject if the symptoms persisted at the afternoon drug dose on day 2. The symptoms were present and the subject reported feeling worse. We discontinued the subject at that point and did not administer the afternoon drug. We obtained a blood sample at that time, made an appointment for the subject with the physician, and arranged for the subject to continue coming in for the next three mornings to allow us to monitor him.

The subject did come in for the monitoring sessions over the next 3 days, and we obtained a final blood sample on the third day. He reported that he was feeling completely "normal" at the first monitoring session (24 hr since last drug intake), and this feeling was reported at all subsequent monitoring

sessions. His pulse rate, blood pressure, and symptom checklist also returned to his pre-drug values. However, he failed to keep his appointment with the physician, and also cancelled a second appointment we had set up for him. He claimed that he felt fine and that it was not necessary to see the doctor. He also stated that he believed the reason he was affected by the drug (he was actually receiving pyridostigmine) was due to his slower-than-normal liver enzyme clearance rate.

It should be noted that this subject was a 4th year medical student who had exhaustively studied pyridostigmine in the PDR. This subject also experienced a claustrophobic reaction while inside the acoustic chamber we are using for the measurement of the Steady State VER. The point of interest here is that one of his hobbies is cave exploration or spelunking. Whether this subject experienced "intern's disease" or a drug effect will be clarified further through analysis of the blood sample taken and further examination of the blood chemistry data taken during the pre-study medical examination.

Sincerely,

Charles Graham, Ph.D.
Principal Investigator

CG/kf

cc: L. Breed, Chairman
MRI Human Subjects Committee

M. Cook, Ph.D., Head
MRI Biobehavioral Sciences Section

cc: Chairman, MRI Human Subjects
Committee
Mary R. Cook

July 20, 1983

Captain Ronald E. Yates
Aerospace Medical Research
Laboratory (AFSC)
AFAMRL/HET
Wright/Patterson AFB, OH 45433

Subject: Contract No. F33615-80-C-0606, MRI Project No. 2030-E(09), "Effects of Pyridostigmine on Psychomotor and Visual Performance."

Dear Captain Yates:

In our letter to you on July 13, 1983, we described the experience of one subject (No. 06) who reported difficulties while taking pyridostigmine. This letter provides additional information on the measures we obtained.

The subject was a medical student who stated that he believed his liver enzymes did not function at the normal clearance rates, and thus, he felt his symptoms might be due to an accumulation of the drug in his system. He based this belief on his observation that drinking one can of beer could make him drunk. If his belief was correct, analysis of his blood cholinesterase levels should show an abnormal reduction under pyridostigmine intake conditions. The following table shows that his levels of cholinesterase differed no more than 5 to 10% between drug and no drug conditions, after taking 4, 30-mg pills of pyridostigmine at 8 hr intervals over a 2-day period. This suggests that his experience with both alcohol and pyridostigmine might be based on something other than biochemistry.

Date	Condition	Cholinesterase Level
6/28/83	Project entry	5238 international units/L
7/1/83	Predrug	5972 international units/L
7/5/83	Drug discontinued	5339 international units/L
7/8/83	Subject reports feeling normal	5751 international units/L

During the course of his participation in the program, we obtained measures of his pulse rate, oral temperature, and blood pressure before, during, and after drug administration. The following table shows the changes observed in these measures. There is a small decrease in pulse rate on day 2 of drug intake; however, all changes seem to be well within the normal range of expected fluctuations.

Date	Time	Condition	Pulse	Temp	BP	
6/29/83	1300	Predrug	60	98.2	130/64	128/66
6/30/83	0823	Predrug	60	97.4	122/72	124/74
7/4/83	0800	Prior to pill 1, day 1	56	96.8	104.68	106.72
7/5/83	0800	Prior to pill 4, day 2	50	97.4	118/68	116/70
7/5/83	1605	Intake discontinued	62	98.2	134/68	134/68
7/6/83	1015	No pill	74	98.1	128/70	132/70
7/7/83	0805	No pill	60	97.8	122/72	118/74
7/8/83	0800	No pill	58	97.2	114/70	114/66

Examination of the data presented above and of the initial medical examination records (enclosed) indicates that Subject No. 06 is basically a healthy young person who may have been more affected by his study of pyridostigmine than by the drug itself.

Sincerely,

Charles Graham, Ph.D.
Principal Investigator

CG/gls

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